6-14-90

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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JUN 14 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review on Cyproconazole

FROM: Esther Rinde, Ph.D. E.R.
Manager, Carcinogenicity Peer Review
Health Effects Division (TS-769c)

TO: Addressees

Attached for your review is a package on Cyproconazole prepared by Dr. Patricia McLaughlin.

A meeting to consider the classification of Cyproconazole is scheduled for Wednesday June 20, 1990, at 10:00 am in Room 821, CM2.

Addressees

P. Fenner-Crisp
W. Burnam
R. Engler
R. Hill
B. Beliles
K. Baetcke
M. Van Gemert
M. Copley
J. Quest
K. Dearfield
H. Pettigrew
W. Sette
G. Ghali
B. Fisher
J. Du
Y. Woo
P. McLaughlin
C. Swenzel

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SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review of Cyproconazole

FROM: Patricia McLaughlin PJ McLaughlin 6/13/90
Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

To: Esther Rinde
Manager, Peer Review for Oncogenicity
Science Analysis and Coordination Branch
Health Effects Division (TS-769)

Through: K. Clark Swentzel K. Clark Swentzel 6/13/90
Section II Head, Toxicology Branch II
Health Effects Division

Background

Cyproconazole is a triazole proposed for use as a turf fungicide. Increased incidences of adenomas and carcinomas were seen in the livers of male and female mice in a long-term feeding study. The Health Effects Division Peer Review Committee has not considered this pesticide. It is requested that this Committee conduct a peer review on this compound and make a recommendation for the classification of an oncogenicity category for cyproconazole. The Committee is also requested to recommend whether or not quantitative risk assessments need to be made for the uses of this pesticide.

Table 1. Liver Tumor Incidence in Cyproconazole Study with CD-1 Mice

DOSES ppm:	0[a]	5	15	100	200
MALES:					
ADENOMA	6/92(7)**[b]	4/49(8)	5/48(10)	12/47(26)*	12/48(25)
CARCINOMA	0/74(0)	0/38(0)	3/46(7)*	3/43(7)**	1/41(2)**
COMBINED	6/92(7)**	4/49(8)	8/48(17)	15/47(32)**	13/48(27)*
FEMALES:					
ADENOMA	0/61(0)**	0/34(0)	0/28(0)	2/41(5)	6/39(15)**
CARCINOMA	0/69(0)**	0/41(0)	0/31(0)	0/43(0)	7/40(18)**
COMBINED	0/69(0)**	0/41(0)	0/31(0)	2/43(5)	13/40(33)**

[a]Composed of two control groups, 50 animals in each one.
[b]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
( ) Percent.
Note: Significance of trend analysis denoted at column for controls; significance of pair-wise comparison with control denoted at dose level. * = $p < 0.05$ and ** = $p < 0.01$.

Table 2. Historical Control Data from Different Sources for Liver Tumors in CR CD-1 Mice

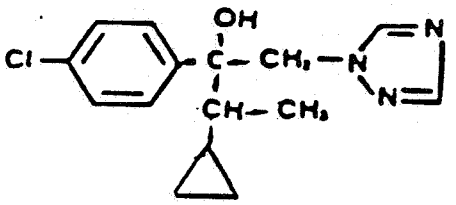
Sex and Tumor Type	24-Month Data Mean %	24-Month Data Range %	18-Month Data Mean %	18-Month Data Range %
Males:				
Adenomas	8.3	0-28	8.2	0-16.3
Carcinomas	6.5	0-21	1.4	0-6
Combined	14.8	5-34		
Females:				
Adenomas	1.7	0-8	1.4	0-2.7
Carcinomas	1.3	0-4	0.2	0-0.7
Combined	3.0	0-10		

B. Material Reviewed

The material available included reviews of the mouse oncogenicity feeding study and the rat chronic toxicity/oncogenicity feeding study. There were also summaries of a one-year dog study, rat and rabbit teratology studies, a two-generation rat reproduction study, mutagenicity, and subchronic studies.

C. Background Information

Cyproconazole is also called SAN 619F. It is alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol, and its Caswell number is 272E. The structure is:



Cyproconazole is a turf fungicide for use on golf courses and sod farms. The registrant is Sandoz Crop Protection Corporation. Cyproconazole is a colorless to brownish powder that is stable and soluble in water.

D. Evaluation of Carcinogenicity Studies of Cyproconazole

1. Long-term Mouse Oncogenicity Study

Reference: Warren, S., Morand de Jouffrey, S., Muller, F., and Karapaly, J.C., May 31, 1989. The Potential Oncogenicity of SAN 619F by Prolonged Dietary Administration to Mice. Study no. 388-M/398-M, Sandoz AG, Agro Division, Basle, Switzerland. MRID No. 411472-01.

Cyproconazole (technical, 95.1% pure) was fed to Charles River CD-1 mice (50/sex/dose group) at concentrations of 5, 15, 100, and 200 ppm. Females were treated for 88 weeks and males for 81 weeks. Ten animals/sex were assigned to control and high dose groups for 13-week interim sacrifice. There were two control groups of 50 animals/sex for the entire study.

Table 1 shows the incidences of liver tumors in male and female mice. There were increased incidences of adenomas, of carcinomas, and of adenomas and carcinomas combined.

In male mice there were statistically significant trends for adenomas and for combined tumors. Males also had statistically significant incidences of carcinomas at the three highest doses, of adenomas at the 100 ppm level, and of combined tumors at the two highest doses.

In female mice there were statistically significant trends for adenomas, for carcinomas, and for combined tumors. Also, all three parameters were statistically significant by pair-wise comparison for the high dose females.

The registrant did not provide historical control data for this strain of mouse from this laboratory. The historical control data available from other sources are shown in Table 2. These data for liver tumors in CD-1 mice are from two sources. The 24-month data are from a series of 36 separate studies at International Research and Development Corporation, where either one or two control groups were used. The second source was a pamphlet published by the Charles River Company (Patricia L. Lang, Spontaneous Neoplastic Lesions in the CRL:CD-1 (ICR)BR Mouse). These 18-month data were from eight groups of control animals in studies that were conducted in different laboratories and completed between 1978 and 1984.

Comparison of Tables 1 and 2 shows that the percent tumors in the following groups in the cyproconazole study exceeded the ranges of percent tumors in the 18-month historical controls: adenomas in 100 ppm males, adenomas in 200 ppm males, carcinomas in 100 ppm males, adenomas in 200 ppm females, and carcinomas in 200 ppm females. The combined tumors in the female mice fed 200 ppm cyproconazole were higher than the range of combined tumors for females in the 24-month historical data. The combined tumor percentages in the male mice fed 100 or 200 ppm cyproconazole were above the mean for combined tumors in 24-month studies.

Male and female mice receiving 100 and 200 ppm cyproconazole had increased incidences of focal hepatocytic inflammation, single-cell necrosis, and diffuse hepatocytic hypertrophy; male mice were more severely affected. Increases in centriacinar hepatocytic vacuolation were seen in high dose males and in mid and high dose females; the mid dose females also had increased periacinar hepatocytic vacuolation. Significant increases in liver weights were seen in both males and females treated with 200 ppm for 13 weeks. At termination, the liver weights of males and females in the two highest doses showed statistically significant increases compared to controls.

There was a decrease in the amount of testicular germinal epithelium in males receiving 200 ppm, which corresponded to the incidence of flaccid testes in this group. Body weight gains were decreased by more than 10% in both sexes fed at 100 and 200 ppm levels for 26 weeks. Mean body weights were significantly decreased in males and females receiving 100 and 200 ppm at 26 and at 52 weeks. In these two groups at termination, the males had 13% lower body weights and the females had 6% lower body weights than the controls. There was no increase in mortality with dose, and no effect of dosing on food consumption or hematology parameters.

Based on the significantly increased incidences of hepatocytic single cell necrosis and diffuse hepatocytic hypertrophy in males and females at 100 and 200 ppm, and a significantly increased incidence of periacinar hepatocytic vacuolation in females at 100 ppm, the NOEL for systemic toxicity is 15 ppm. Fifteen ppm corresponds to an intake of approximately 1.8 mg/kg/day in males and 2.6 mg/kg/day in females.

2. Two-year Rat Chronic/Oncogenicity Study

Reference: Warren, S.F.P., Carpy, S., and Muller, F., April 22, 1988. San 619F Chronic Toxicity/Oncogenicity Feeding Study in Rats. Study No. 357-R, Sandoz Ltd. Agro Development, Basle, Switzerland. MRID No. 411647-01.

Cyproconazole (technical, 94.6-96.6 % purity) was fed to 70 KFM-Wistar rats per sex per group at 20, 50, and 350 ppm. These levels correspond to intakes of 1.0, 2.2, and 15.6 mg/kg/day for males and 1.2, 2.7, and 21.8 mg/kg/day for females. Ten rats/sex/dose were sacrificed at 12 and at 18 months.

There were decreased body weights in high dose females, although generally less than 10 percent different from control values, and an increased incidence of fatty infiltration of the liver in the high dose males. The liver findings are listed in Table 3. Based on these effects, the LOEL for systemic toxicity was 350 ppm and the NOEL was 50 ppm.

The study did not show carcinogenicity. However, the lack of: 1) any biologically significant body weight decrement; 2) any significant histopathological correlate accompanying the increased relative liver weight; 3) any increase in the liver enzyme activities in the females; and 4) any consistent change in the liver enzyme activities in the high dose males, all suggest that the dose levels chosen were not adequate to determine the carcinogenic potential of the test material.

Table 3. Liver Effects in the Chronic Rat Study

	Males				Females			
Doses ppm:	0	20	50	350	0	20	50	350
No. examined[a]	50	49	50	50	50	50	50	50
Fatty change	23	19	29	38	23	15	15	10
Hyperplastic nodule	3	3	0	2	3	1	1	2
Bile duct proliferation	22	23	19	26	27	35	32	38
Sinusoidal cell pigmentation	1	1	4	5	5	7	6	13
Vacuolated focus	14	18	19	16	2	9	9	9
No. examined[b]	50	50	50	50	50	50	50	50
Adenoma	1	0	1	1	0	0	1	1
Carcinoma	0	0	1	1	0	1	0	0

[a]Includes animals in the main study that died or were sacrificed moribund as well as those sacrificed at study termination.
[b]Includes all rats in the main groups.

E. Additional Toxicology Information

1. Mutagenicity

Cyproconazole has been tested in several mutagenicity studies considered acceptable by the agency. Ames tests in S. typhimurium were negative with and without activation. There was no in vitro cell transformation with Syrian hamster embryo cells, with and without activation. HGPRT gene mutation tests in Chinese hamster ovary cells were negative with and without activation. A micronucleus assay in mice was negative.

An assay for potential to induce chromosome aberrations in Chinese hamster ovary cell was positive in nonactivated and activated conditions. These results indicate that cyproconazole can be clastogenic without metabolic activation.

2. Metabolism

No metabolism data are available for cyproconazole. However, the liver effects, such as were seen in the one-year dog study and the chronic rat study, are consistent with the metabolism of cyproconazole being like that of structurally similar compounds (see part 5 below).

3. Acute, Subchronic, and Chronic Toxicity

The acute oral LD50 in rats was over 1000 mg/kg.

Effects associated with treatment in a thirteen-week rat feeding study (20, 80, 320 ppm, with a recovery period) were inhibited body weight gain, increased blood sodium, and increased liver weights at the high dose. The high dose also had histological changes in the liver, that is, vacuolated hepatocytes and a distinct lobular pattern associated with enlarged hepatocytes. Blood creatinine was increased and calcium was decreased at the high and low doses, but not at the mid dose; thus this study did not attain a NOEL.

Changes associated with treatment in a thirteen-week dog feeding study included, at the high dose of 500 ppm, slack muscle tone, inhibited body weight gain, and decreases in bilirubin, total cholesterol, HDL-cholesterol, triglycerides, total protein, and albumin. There were increases in platelet counts, alkaline phosphatase, gamma glutamyl transferase, absolute and relative liver weights, relative kidney weights, and relative brain weights. Liver toxicity was shown by hepatocytomegaly, degeneration of single hepatocytes, and cytoplasmic inclusions. The mid-dose (100 ppm) dogs had increased absolute liver weights and hepatocytomegaly. The LEL was 100 ppm and the NOEL was 20 ppm.

Effects on the liver in a one-year dog feeding study were indicated by elevated alkaline phosphatase and ALAT levels; decreased total protein, albumin, and cholesterol levels. Absolute and relative liver weights were increased. Relative kidney weights were increased in low and high dose females and cytochrome P450 was increased in mid and high dose animals. Laminar eosinophilic intrahepatocytic bodies were observed in high dose animals. The NOEL was set at 30 ppm and the LEL at 100 ppm based on liver effects.

4. Developmental and Reproductive effects

A teratogenicity study in rats (6, 12, 24, and 48 mg/kg/day on gestation days 6-15) showed dose related increases in the number of fetuses with supernumerary ribs at all doses. The two highest doses showed decreased total number of fetuses/dam, decreased number of live fetuses/dam, increased fetal resorptions, decreased body weight, and incomplete or absent ossification in some foot bones. There were instances of hydrocephaly and cleft palate at the two highest doses. There were maternal body weight differences, but the influence of treatment-related intrauterine effects on maternal weight make the evidence for maternal toxicity, and the maternal NOEL of 6 mg/kg, equivocal. The developmental NOEL was 6 mg/kg and the LEL 12 mg/kg.

In a rabbit teratology study (2, 10, 50 mg/kg/day on gestation days 6-18) a developmental NOEL was not attained. Hydrocephalus internus was found at all doses and agenesis of the kidney and ureter occurred at the high dose. Also at the high dose there was a decreased number of live fetuses/dam and an increased incidence of non-ossification in some digits. Resorptions increased at the mid and high dose. The maternal NOEL of 10 mg/kg and LEL of 50 mg/kg, based on inhibited body weight gain and decreased food consumption, are equivocal. This study was classified as supplementary.

These studies will be assessed by the HED Developmental Toxicity Peer Review Committee.

A two generation rat reproduction study (4, 20, and 120 ppm cyproconazole in the diet) showed liver toxicity by increased lipid storage and relative liver weight. Longer gestation (at high and mid dose) and fewer implantation sites (at high dose) were seen. There were decreases in litter sizes (in high and mid-dose), live birth index (high dose), and viability index (high dose). The LEL was 20 ppm and the NOEL 4 ppm.

5. Structure-Activity Relationships

Cyproconazole is structurally similar to other triazole pesticides such as hexaconazole, triadimefon (Bayleton), triadimenol (Baytan), propiconazole (Tilt), uniconazole (Prunit), terbuconazole (Folicur), etaconazole (Sonax, Vanguard), bitertanol (Baycor), and azaconazole. These compound structures are in Figure 1.

Bayleton was associated with increased incidences of hepatocellular adenomas in male and female mice when administered in diet at a concentration of 1800 ppm. No increase in liver carcinomas was reported.

Triadimenol, a primary and major metabolite of Bayleton, was classified by the HED Peer Review Committee in their meeting of October 1, 1987 as group C, a possible human carcinogen, based upon increased incidences of liver adenomas in female CF1-W74 mice (HED Report dated January 29, 1988). This classification was upheld by the FIFRA Scientific Advisory Panel in their meeting of December 15, 1987 (report dated December 23, 1987).

Propiconazole was associated with increased incidences of hepatocellular adenomas and carcinomas in female CD-1 mice, and was classified by the HED Peer Review Committee in group C, possible human carcinogen (HED Reports dated April 29, 1987, July 21, 1988, April 28, 1989, and January 22, 1990).

Etaconazole was associated with increased incidence of liver adenomas and carcinomas in both male and female Albino Swiss mice. However, the registration application was voluntarily withdrawn by the registrant and therefore no further action was taken regarding its cancer classification.

Uniconazole, a new pesticide currently under evaluation, was associated with statistically significant increases in the incidence of liver adenomas and adenomas/carcinomas combined in male CD-1 mice.

Bitertanol and azaconazole were reported to be negative for carcinogenicity in mice when administered in the diet up to 180 and 200 ppm, respectively. With the exception of cyproconazole and hexaconazole, all other triazole fungicides were tested at dietary concentrations of 1500 ppm or higher before inducing any carcinogenic response in mice.

Hexaconazole was associated with increased incidence of benign Leydig cell tumors in the testes of rats, a tumor type not previously reported in response to compounds of this type. In addition, no treatment related liver tumors were noted in mice; however, the highest dietary concentration used, 300 ppm, was well below the concentration where hepatocellular tumors occurred in response to other compounds.

Most of these triazole pesticides were associated with some developmental toxicity, particularly skeletal variations and malformations. For example, bitertanol, bayleton and cyproconazole were reported to induce cleft palates, a rare effect in rats. Triadimenol, terbuconazole and hexaconazole, along with cyproconazole, were associated with increased incidence of supernumerary ribs in rats.

F. Weight of Evidence Considerations

The committee considered the following facts regarding the toxicology data on cyproconazole to be of importance in a weight-of-the-evidence determination of carcinogenic potential.

1. Administration of cyproconazole was associated with an increased incidence of hepatocellular tumors in male and female CD-1 mice. At a dietary dose level of 200 ppm, incidences of carcinomas and of combined adenomas and carcinomas were significantly increased in both sexes. Also at this dose, females had a significantly increased incidence of adenomas. Males had significantly increased incidences of adenomas, of carcinomas, and of both tumor types combined at 100 ppm dose of cyproconazole, as well as of carcinomas at 15 ppm dose.

2. In both the female and male mice, there were statistically significant trends for adenomas and for combined tumors. Female mice showed a statistically significant trend for carcinomas, also.

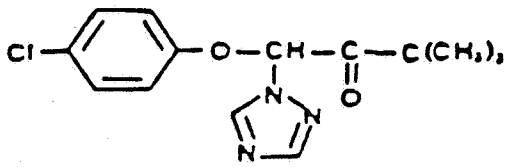
3. The increased incidences of hepatocellular tumors noted at 100 and 200 ppm in male CD-1 mice and at 200 ppm in female CD-1 mice generally exceeded the available historical control incidences for these same tumor types in mice of this strain. However, the available historical control data were generated at other laboratories under different conditions and at different time periods. Some of these studies were of longer duration than the cyproconazole study. In these circumstances, the historical control data are considered ancillary information and only minimally confirmatory in nature.

4. There was no compound-related increase in tumors observed in male or female rats. However, it appears that the dose levels fed in the long term rat study were not adequate to determine the carcinogenic potential of the test material.

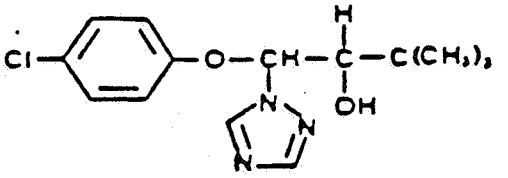
5. Although several genetic toxicity tests were negative, an assay on induction of chromosome aberrations in Chinese hamster ovary cells was positive in nonactivated and activated conditions. These results indicate that cyproconazole can be clastogenic without metabolic activation.

6. Cyproconazole dosing was associated with teratogenicity in the form of hydrocephaly and cleft palate in rats and of hydrocephalus internus and agenesis of the kidney and ureter in rabbits. There were also supernumerary ribs in rats and incomplete or absent ossification in some foot bones in both species.

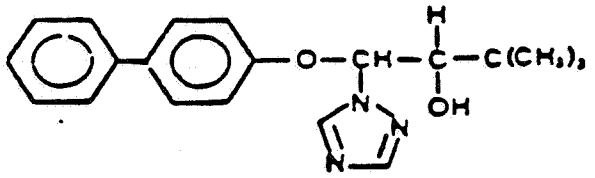
7. Cyproconazole is structurally related to several triazole pesticide compounds, most of which have been associated with the formation of liver adenomas or carcinomas. Also, most of these triazoles were associated with developmental toxicity, particularly of the skeleton.



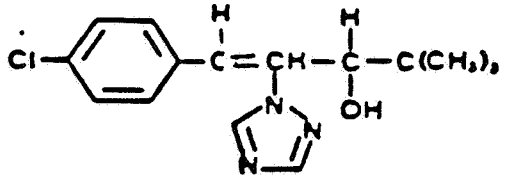
Triadimefon
(Bayleton)



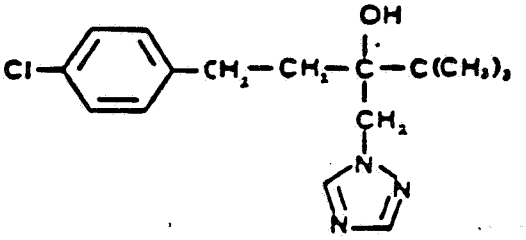
Triademenol
(Baytan)



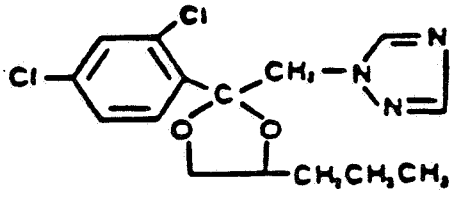
Bitertanol
(Baycor)



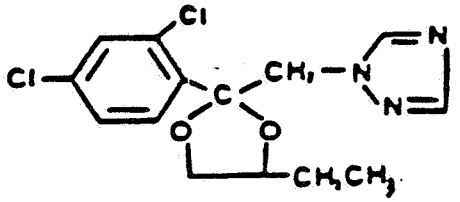
Uniconazole
(Prunit)



Terbuconazol
(Folicur)

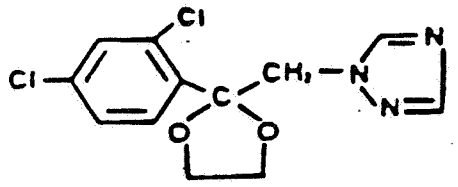


Propiconazole
(Tilt)

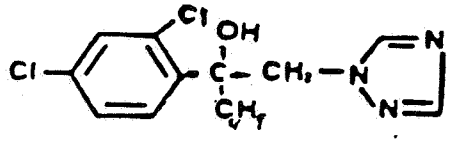


Etaconazole
(Vangard)

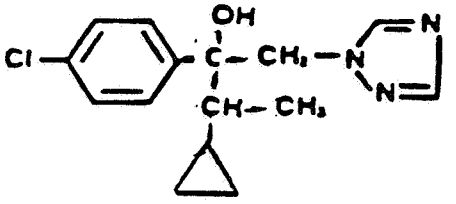
Figure 1. Structurally Related Compounds



Azaconazole



Hexaconazole
(Anvil)



Cyproconazole
(SAN 619F)

Figure 1. Structurally Related Compounds

EPA No.: 68D80056
DYNAMAC No.: 244-A
TASK No.: 2-44A
November 17, 1989

7-1768

DRAFT

DATA EVALUATION RECORD

CYPROCONAZOLE (SAN 619F)

Oncogenicity Feeding Study in Mice

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: _____

Date: _____

pages 4-5, 6, 7, 10, 11, 12, 15, 17, 18 19, 20, 21, 23, 24

call if you have any questions
LLT
557-0486

Sent back
5-30-90

(20)

EPA No.: 68D80056
DYNAMAC No.: 244-A
TASK No.: 2-44A
November 17, 1989

DATA EVALUATION RECORD

CYPROCONAZOLE (SAN 619F)

Oncogenicity Feeding Study in Mice

REVIEWED BY:

John J. Liccione, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: _____
Date: _____

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

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Date: _____

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Date: _____

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EPA Reviewer, Section II
Toxicology Branch (H-7509C)

Signature: Linda Lee Taylor C
Date: 5-30-90

K. Clark Swetzel, ~~Ph.D.~~
EPA Section Head, Section II
Toxicology Branch II
(H-7509C)

Signature: _____
Date: _____

DATA EVALUATION RECORD

GUIDELINE §83-2

STUDY TYPE: Oncogenicity feeding study in mice.

MRID NUMBER: 411472-01.

TEST MATERIAL: Cyproconazole (SAN 619F).

SYNONYM(S): Alto; cyproconazol; 2-(4-chlorophenyl-3-(1H-1,2,4-triazol-1-Yl)butan-2-01.

STUDY NUMBER(S): Project 388-M/398-M.

SPONSOR: Sandoz Crop Protection Corporation, Des Plaines, IL.

TESTING FACILITY: SANDOZ AG, Agro Division, Dept. of Toxicology, Basle, Switzerland.

TITLE OF REPORT: The Potential Oncogenicity of SAN 619F by Prolonged Dietary Administration to Mice.

AUTHOR(S): Warren, S., Morand de Jouffrey, S., Müller, F., and Karapaly, J. C.

REPORT ISSUED: April 24, 1989 (reissued May 31, 1989).

CONCLUSIONS:

Cyproconazole was fed to CD-1 mice at dietary levels of 5, 15, 100, or 200 ppm for 81 weeks (males) or 88 weeks (females). There were increased incidences of combined adenomas and carcinomas in the livers of males receiving 15, 100, and 200 ppm, and in females receiving 200 ppm. ~~The increased incidence of hepatocytic tumors in males of the 15-ppm dose group was not considered treatment related but rather due to increased survival among these mice.~~ according to Q.A. Tox
Male and female mice receiving 100 and 200 ppm had increased incidences of focal hepatocytic inflammation, single-cell necrosis, and diffuse hepatocytic hypertrophy; male mice were more severely affected. There was a decrease in the amount of testicular germinal epithelium in males receiving 200 ppm. The increased testicular germinal epithelial deficit corresponded to the increased incidence of flaccid testes in male mice of the 200-ppm dose group. Body weight gains were decreased by more than 10% in both sexes fed cyproconazole at dietary levels of 100 and 200 ppm for 26 weeks. Significant increases in liver weights were seen in both males and females treated with 200 ppm cyproconazole in the diet for 13 weeks. There was no effect of dosing on mortality, food consumption, or hematology parameters. Based on a significantly increased incidence of hepatocytic single cell necrosis and diffuse hepatocytic hypertrophy in male and female mice receiving 100 and 200 ppm, and a significantly increased incidence of periacinar hepatocytic vacuolation in female mice receiving 100 ppm, the NOEL for systemic toxicity is 15 ppm, corresponding to an intake of approximately 1.8 mg/kg/day in males and 2.6 mg/kg/day in females.

Classification: Core Guideline.

A. MATERIALS:

1. Test Compound: Cyproconazole technical (SAN 619-F); description: colorless crystals; batch No.: 8507; purity: 95.1%.

2. Test Animals: Species: mouse; strain: CD-1; age: 28 days; weight: mean body weights at initiation of the main study--30 g (males) and 22 g (females); source: Charles River (Wiga) Breeding Laboratories, D-8741 Sulzfeld, BRD.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimated to laboratory conditions for 15 days and were assigned by sex to the following test groups using computer-generated randomization:

Test group	Dietary Level (ppm)	Main study (88 weeks)[a] Males	Females	Interim sacrifice (13 weeks) Males	Females
1	0[c] (Control-1)	50	50	10	10
2	5	50	50	-	-
3	15	50	50	-	-
4	100	50	50	-	-
5	200	50	50	10	9[b]
6	0[c] (Control-2)	50	50	-	-

[a]Females were treated for 88 weeks, males for 81 weeks.

[b]One female from group 5 was used to replace a mis-sexed main group animal at the start of the study, leaving only nine in the high-dose female group.

Mice were housed individually in cages in a room with temperature and humidity controls set at 23°C and 50%, respectively, and with a 12-hour light/dark cycle. No formal health screening was performed prior to treatment. However, after animals died in a neighboring room during week 46, blood samples were drawn from the orbital sinus, under light ether anesthesia, from the first six mice in each room and assayed for virus antibody titres. In addition, blood samples were drawn immediately postmortem during weeks 71-74 from mice killed in extremis, and during week 82 from male mice killed at termination, and analyzed for antibody titres.

2. Diet Preparation: Diets (100- and 200-ppm) were prepared by diluting a 1% premix with the appropriate amount of untreated diet to give the desired concentration. The 1% premix was also used to prepare a premix of 1000 ppm, which was then used to prepare diets of 5 or 15 ppm by direct dilution. The homogeneity and stability of dietary samples were determined in a previous study. During the study, samples of each premix were analyzed for accuracy of mixing at monthly intervals, and samples of each final diet were analyzed at least each 2 months.

Results: Table 1 summarizes data on nominal and analyzed dietary levels of cyproconazole. Values for test compound were generally in agreement with nominal values. The percentage deviation from nominal values, determined at various intervals throughout the treatment period, were -20% to +36%, -13% to +27%, -16% to +23%, and -22% to +6% at dose levels of 5, 15, 100, and 200 ppm, respectively.

TABLE 1. Dietary Levels of Cyproconazole

Nominal Level (ppm)	Analyzed Level	
	ppm	Range
5	5.2 ± 0.~~799~~ 8	(4.0 - 6.3)
15	15.9 ± 1.~~66~~ 7	(13.0 - 19.0)
100	100.6 ± 10.4	(84.0 - 123.0)
200	192.4 ± 14.5	(174.0 - 206.0)

OK

(25)

3. Food and Water Consumption: Animals received KLIBA powdered diet No. 32-343-4 (designated 21-343-4 until week 40) and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data. One-way analysis of variance, followed by a multiple comparison to controls using Dunnett's test, was used for comparison of controls and dosed groups when variance was homogeneous. The Kruskal-Wallis test was used when variance was not homogeneous and when more than one treated group was present. The Dunn-Bonferroni test was used to perform a multiple comparison to the control group if a significant difference existed between the groups. The Mann-Whitney U test was performed for samples of four or more animals per group when only one treated group was present; with three or fewer animals per group, the Student's t-test was used.

   Adjusted mortality rate was calculated by the method of Kaplan and Meier, and intergroup differences in mortality were analyzed with Cox's method using the Tarone test for linear trend. Age-adjusted tumor analysis was essentially performed by the method of Peto, analyzing fatal tumors with lifetable analysis, incidental tumors by prevalence analysis and all tumors by combined analysis.

5. Quality Assurance: A quality assurance statement was signed and dated May 26, 1989; an updated GLP compliance statement was also present. A separate quality assurance statement for the final pathology report was dated May 30, 1989.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of mortality and for gross signs of ill-health or reaction to treatment. Detailed examinations were performed biweekly from week 66 and weekly from week 78.

   Results: Cumulative mortality and survival data are summarized in Table 2. Mortality of mice receiving 100 or 200 ppm was less than that of the controls or mice receiving 5 or 15 ppm. Statistical analysis of mortality (Tarone's one-tailed test) indicated a negative trend for males ($p < 0.001$) and females ($p = 0.004$). By pairwise comparison, there was a significantly increased survival compared to controls in males receiving 15 and 100 ppm ($p < 0.05$) and 200 ppm ($p < 0.001$); for females, a significant effect ($p < 0.05$) was noted at 100 ppm but not

? 0.01

(24)

TABLE 2. Cumulative Mortality and Percent Survival in Mice Fed Cyproconazole for 88 Weeks[a,b]

Dose Group (ppm)	Mortality (Percent Survival) at Week: Satellite Groups 13	Main Groups 52	Main Groups 80	Main Groups Termination[a]
		Males		
0	10(100)	4(92)	30(40)	31(38)
5	--	1(98)	29(42)	30(40)
15	--	1(98)	19(62)	25(50)
100	--	2(96)	21(58)	21(58)
200	10(100)	1(98)	11(78)	14(72)
0	--	3(94)	32(36)	34(32)
		Females		
0	10(100)	2(96)	25(50)	33(34)
5	--	0(100)	18(64)	26(48)
15	--	2(96)	24(52)	30(40)
100	--	2(96)	9(82)	14(72)
200	9(100)[c]	5(90)	12(76)	23(54)
0	--	2(96)	14(72)	27(46)

[a]Female mice were treated for 88 weeks, males for 81 weeks; terminal sacrifices were performed during week 90 for females and week 82 for males.

[b]Percent survival was based on 50 mice/sex/dose in the main group and 10 mice/sex/dose in the satellite group.

[c]One group 5 female mouse was used to replace a mis-sexed main group mouse at the start of the study, leaving only nine in the high-dose female group.

at 200 ppm. There were no deaths among the 39 mice allocated to the satellite group.

Serology samples were drawn during week 46 as a response to unexpected, sudden mortality in an adjacent room. Results showed the presence of antibodies to mouse hepatitis virus (MHV) in an unspecified number of six mice tested from each room; however, no symptoms of ill health were observed in the study, and the putative infection could be shown to be present only as a result of seroconversion. Blood samples were also drawn from mice killed in extremis during weeks 71 to 74; these were compared with samples from male mice killed at termination at week 82 to determine whether virus antibody titres had increased in mice that had died spontaneously, which might have indicated an adverse effect on survival due to the infection. These samples again revealed seroconversion to MHV (19/29 of mice tested). Seroconversion of 16/29 mice to Mycoplasma pulmonis, and of 1/29 mice to Sendai virus, was also noted. However, no pattern of incidence was seen of seroconversion between mice killed in extremis or killed at termination, or between mice in the two different rooms in which the study was housed.

The study authors concluded that the presence of seroconversion to the agents detected did not correlate with an adverse effect on the study due to these pathogens.

2. Body Weight: Mice were weighed before treatment and once each week thereafter.

Results: Table 3 summarizes data on mean body weights at selected intervals. Mean body weights were significantly decreased ($p < 0.01$) in males and females receiving 100 and 200 ppm at weeks 26 and 52. At termination, mean body weights in males receiving 100 and 200 ppm were 13% lower ($p < 0.01$) than controls; in females receiving the same doses, the body weights were decreased ($p < 0.05$) by 6%. Table 4 summarizes data on mean body weight gains between 0 and 26 weeks. Body weight gains were significantly decreased by more than 10% in both sexes receiving 100 and 200 ppm for 26 weeks.

3. Food Consumption and Compound Intake: Food consumption was determined weekly throughout the study duration. Compound intake was calculated from the consumption and body weight gain data and from nominal dietary concentrations.

Results: No treatment-related effects on food consumption were seen. A statistically greater food consumption (4.4% greater than control) among male mice receiving 5 ppm during the first 26 weeks of treatment was not considered to be of toxicological significance. Mean compound intake

TABLE 3. Mean Body Weight at Selected Intervals in Mice Fed Cyproconazole for 88 Weeks[a]

Dietary Level (ppm)	Mean Body Weights (g ± S.D.) at Week: 0	26	52	81
		Males		
0	30 ± 2	42 ± 5	44 ± 5	46 ± 8
5	30 ± 2	40 ± 5	42 ± 5	44 ± 5
15	29 ± 1	40 ± 4	43 ± 5	44 ± 6
100	30 ± 2	38 ± 4**	40 ± 4**	40 ± 5**
200	30 ± 1	38 ± 4**	41 ± 5**	40 ± 4**
0	30 ± 2	42 ± 5	44 ± 5	44 ± 7
		Females		
0	22 ± 1	29 ± 2	32 ± 3	33 ± 4
5	22 ± 1	29 ± 2	31 ± 3	31 ± 3*
15	22 ± 2	30 ± 3	32 ± 3	33 ± 5
100	22 ± 2	28 ± 2*	30 ± 2**	31 ± 3*
200	22 ± 2	28 ± 2**	30 ± 3**	31 ± 3*
0	22 ± 2	29 ± 3	32 ± 4	32 ± 3

[a]Females were treated for 88 weeks, males for 81 weeks.

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

TABLE 4. Mean Body Weight Gains in Mice Fed Cyproconazole for 26 Weeks

Dietary Level (ppm)	Mean Weight Gain (gram gain/mouse/week) Between 0 and 26 Weeks	
	Males	Females
0 (control-1)	0.44	0.27
5	0.41 (-7.8)[a]	0.25 (-5.7)
15	0.41 (-8.0)	0.30 (+10.8)
100	0.32** (-28.3)	0.22** (-19.2)
200	0.32** (-28.7)	0.22* (-17.7)
0 (control-2)	0.44 (-1.3)	0.27 (-0.7)

[a]The values in parentheses are the percent decreases in mean weight gain compared to controls.

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

values for 81 weeks (males) or 88 weeks (females) of the study were 0.69, 1.84, 13.17, and 27.85 mg/kg/day for males and 1.03, 2.56, 17.65, and 36.30 mg/kg/day for females receiving 5, 15, 100, or 200 ppm, respectively.

4. Ophthalmological Examinations: Ophthalmological examinations were not performed.

5. Hematology and Clinical Chemistry: Blood smears were prepared after 52 weeks, after 79 weeks, and prior to female terminal sacrifice (90 weeks) from mice of the control group and from the 200-ppm group for differential white blood cell count. The following cell types were evaluated by light microscopy: banded neutrophils, segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Clinical chemistry parameters were not examined.

   Results: No effects of dosing on leukocyte differential count were seen.

6. Urinalysis: Not performed.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination; the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

Digestive System	Cardiovasc./Hemat.	Neurologic
X Tongue	Aorta†	XX Brain
X Salivary glands†	XX Heart†	X Peripheral nerve (sciatic nerve)†
X Esophagus†	X Bone marrow†	X Spinal cord (3 levels)
X Stomach†	X Lymph nodes†	X Pituitary†
X Duodenum†	XX Spleen	X Eyes (optic nerve)†
X Jejunum†	X Thymus	
X Ileum†		
X Cecum†		
X Colon†		
X Rectum†		
XX Liver†		
X Gallbladder†		
X Pancreas†		

Urogenital	Glandular
XX Kidneys†	XX Adrenals†
X Urinary bladder†	Lacrimal gland
XX Testes†	X Mammary gland†
X Epididymes	X Thyroids†
X Prostate	X Parathyroids†
X Seminal vesicle	Harderian glands
X Ovaries	
X Uterus	
X Cervix	

Respiratory	X Other
X Trachea†	X Bone (sternum and femur)†
X Lung†	X Skeletal muscle†
	X Skin
	X All gross lesions and masses

†Recommended by Subdivision F (October 1982) Guidelines.

Results:

a. Organ Weights: After 13 weeks of treatment, the liver weights of male and female mice receiving 200 ppm were significantly increased ($p < 0.01$) when compared to controls for both the absolute weight and the weight relative to body weight. At termination, the liver weights of males and females receiving 100 or 200 ppm were again significantly increased ($p < 0.01$) when compared to controls. Following an analysis in which the weights of livers with a tumor greater than 10 mm diameter were excluded, the study authors concluded that the increase in liver weight was not a consequence of neoplasia. There were no other organ weight changes that could be considered a result of treatment. Table 5 presents liver weight data.

b. Gross Pathology: Table 6 summarizes the incidence of frequently observed gross lesions in mice sacrificed or dying during the treatment period of the main study. A significantly increased incidence of hepatic accentuated lobular pattern was found among males treated with 15, 100, and 200 ppm cyproconazole, and among females treated with 100 and 200 ppm. The incidence of hepatic masses was also increased in male mice of the 100-, and 200-ppm dose group, and in female mice treated with 200 ppm. The hepatic masses corresponded to the treatment-related increases in hepatic adenomas and carcinomas in both sexes. There were also increased incidences of nonspecified areas of hepatic change and enlargement in female mice of the 200-ppm dose group, and of "granular" livers in the corresponding male group. Significantly increased incidences of flaccid testes were noted in male mice of the 15- and 200-ppm dose groups; however, these increased incidences corresponded to an increased testicular germinal epithelial deficit in male mice of the 200-ppm dose group only. Increased incidences of skin ulceration observed in all treated groups of both sexes were significantly different only in female mice of the 200-ppm dose group. Significant reductions in the incidences of edema of the subcutis were reported in male mice treated with 100 and 200 ppm. Renal findings that may relate to treatment were restricted to a reduced incidence of granular kidneys in females receiving 200 ppm, and to an apparent reduction in kidney size of the corresponding male group. A number of other gross findings not considered related to dosing were reported. These findings included increased incidences of apparent heart auricle enlargement in males, reduced incidence of cystic

TABLE 5. Mean Liver Weights (g ± S.D.) and Liver Weights as a Percentage of Body Weight in Mice Fed Cyproconazole for 88 Weeks[a]

Dietary Level (ppm)	Liver Weights at Time of Sacrifice			
	Interim Sacrifice (Week 14)			
	Males		Females	
	(g)	(%)	(g)	(%)
0	1.355 ± .283	3.988 ± .372	1.045 ± .136	4.410 ± .582
200	1.913 ± .314**	6.097 ± .547**	1.511 ± .467**	6.425 ± 1.51**
	Terminal Sacrifice			
	Males		Females	
	(g)	(%)	(g)	(%)
0	2.554 ± 1.350	5.488 ± 2.634	1.730 ± 0.251	4.963 ± 0.479
5	2.193 ± .248	4.885 ± 0.630	1.536 ± 0.221	4.878 ± 0.560
15	2.426 ± .446	5.322 ± 1.028	1.828 ± 0.669	5.257 ± 2.077
100	2.770 ± .884	6.682 ± 1.769**	2.145 ± 0.384**	6.407 ± 0.932**
200	3.228 ± 1.242**	7.789 ± 2.602**	2.570 ± 0.621**	7.897 ± 1.692**
0	2.252 ± 0.500	5.108 ± 1.144	1.648 ± 0.194	4.895 ± 0.544

[a]Females were treated for 88 weeks, males for 81 weeks.

**Significantly different from control value ($p < 0.01$).

TABLE 6. Representative Gross Findings in Mice Fed Cyproconazole for 88 Weeks[a,b]

Organ/Finding	Males						Females					
	Control Group 1: 0	5	15	100	200	Control Group 2: 0	Control Group 1: 0	5	15	100	200	Control Group 2: 0
Heart, auricle												
Enlarged	11	9	19**	6	8	7	2	2	5	2	5	2
Kidneys												
Granular	14	16	17	13	12	13	21	21	17	17	10*	18
Cystic	14	14	14	5*	9	11	7	7	4	9	3	8
Liver												
Accentuated lobular pattern	3	5	10*	13***	23***	3	3	4	3	12**	21***	4
Masses	5	5	8	17***	20***	5	1	1	3	5	18***	2
Granular	0	0	0	1	4*	1	1	0	1	0	3	1
Enlarged	1	0	1	2	5	3	2	1	1	6	10**	2
Skin												
Ulceration	6	7	7	7	10	3	2	6	4	6	9*	4
Subcutis Edematous	8	12	11	3*	2*	12	6	3	6	0*	5	4
Testes												
Flaccid	1	4	9**	6	9**	3	0	0	0	0	0	0

[a]Females were treated for 88 weeks, males for 81 weeks.

[b]Includes all animals in main groups (50/sex/group), but not interim sacrifice animals.
*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

***Significantly different from control value ($p < 0.001$).

kidneys in males, increased incidence of seminal vesicle enlargement, increased skin pallor in males, and a reduction in perineal staining in females. No significant pathological changes were detected among mice in the satellite study.

c. Microscopic Pathology:

1) Nonneoplastic: The only nonneoplastic effects observed at the interim sacrifice, and that were attributed to dosing, were in the liver. The incidence of periacinar hepatic hypertrophy was increased in the high-dose males (10/10) and females (8/10) when compared to controls (males--4/10, females--0/10). In addition, periacinar hepatic vacuolation was seen in five males and non-zonar hepatocytic vacuolation in four males and eight females. Hepatocytic vacuolation was not seen in any of the controls at the interim sacrifice (week 14). Table 7 summarizes the incidences of frequently occurring nonneoplastic lesions in mice in the main group, combining data for those that died, were sacrificed moribund, or were sacrificed at termination. The primary treatment-related alterations following oral treatment with cyproconazole were seen in the liver. Increased incidences of focal hepatocytic inflammation, single-cell necrosis, and diffuse hepatocytic hypertrophy were noted in both male and female mice receiving 100 and 200 ppm. An increased incidence of centriacinar hepatocytic vacuolation was seen in males receiving 200 ppm and in females receiving 100 and 200 ppm. An increased incidence of periacinar hepatocytic vacuolation was seen in females treated with 100 ppm. A number of significant but trivial changes were also reported. In male mice receiving 100 and/or 200 ppm, these changes included increased incidences of epididymal aspermia, optic nerve gliosis, skin ulceration, cellulitis, and testicular epithelial deficit. Decreased incidences of pancreatic edema and subcutaneous edema, and interstitial degeneration in the salivary glands, were also seen in these males. In female mice receiving 100 and/or 200 ppm, increased incidences of aortic arteritis and lymphoid hyperplasia in the mesenteric lymph nodes, and decreased incidences of subcutaneous edema and spinal cord compression, were reported. Slight to

TABLE 7. Representative Nonneoplastic Finding in Mice Fed Cyproconazole for 88 Weeks[a,b]

Organ/Finding	Dietary Level (ppm)											
	Males						Females					
	0	5	15	100	200	0	0	5	15	100	200	0
Liver	(50)[c]	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Focal inflammation	1	1	4	5*	8**	1	1	5	9	5	4	6
Single cell necrosis	0	2	3	14**	25***	2	0	3*	2	4*	9***	0
Diffuse hepatocytic hypertrophy	4	4	6	26***	36***	10	5	6	6	7	20***	8
Centriacinar hepatocytic vacuolation	0	0	0	1	3*	0	0	0	0	4*	3*	0
Periacinar hepatocytic vacuolation	0	4*	3	1	1	1	0	0	1	17**	6**	1
Periacinar hepatocytic hypertrophy	1	2	5*	4*	2	0	0	1	0	0	3*	0
Focal hepatocytic hyperplasia	1	0	2	2	2	1	0	0	1	0	5**	0
Testes	(50)	(50)	(50)	(50)	(50)	(50)	(0)	(0)	(0)	(0)	(0)	(0)
Germinal epithelium deficit	22	31	29	34**	33*	23	0	0	0	0	0	0
Optic nerve	(45)	(45)	(44)	(45)	(46)	(41)	(40)	(40)	(43)	(38)	(37)	(39)
Gliosis	0	2	2	2	3*	0	0	2	0	1	2	1
Salivary gland	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Interstitial degeneration	8	6	1	0*	0*	2	3	4	0	0	0	0
Skin	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Ulceration	4	5	4	6	9*	2	1	4	3	5	4	3
Cellulitis	4	5	6	8*	8*	1	1	4	3	5	6	3
Subcutaneous edema	7	5	12	3	2*	11	6	4	6	1*	1*	11
Epididymides	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Aspermia	10	20	15	26**	21*	15	0	0	0	0	0	0
Heart, ventricle	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(50)	(50)	(50)	(50)	(50)
Aortic arteritis	0	0	0	0	0	1	1	0	0	2	4*	0
Mesenteric lymph node	(48)	(45)	(49)	(49)	(47)	(50)	(47)	(49)	(46)	(50)	(50)	(50)
Lymphoid hyperplasia	0	0	2	2	0	1	3	2	1	2	13*	7

(continued)

TABLE 7. (continued)

Organ/Finding	Dietary Level (ppm)											
	Males						Females					
	0	5	15	100	200	0	0	5	15	100	200	0
Pancreas	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Edema	3	12	8	1	0*	9	6	6	7	2	2	4
Caecum	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Submucosal edema	5	7	11	4	6	12	7	7	8	3*	0**	12
Spinal cord (cervical)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(50)	(50)	(50)
Cord compression	6	3	5	2	4	2	5	1	5	2	0*	6

[a]Female mice were treated for 88 weeks, males for 81 weeks.

[b]Fisher's two-tailed Exact test comparing pooled controls vs. the treated groups was used for statistical evaluation of the data.

[c]The numbers in parentheses are the numbers of animals with specific organ examined microscopically.

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

***Significantly different from control value ($p < 0.001$).

moderate amyloidodis was observed in both sexes, with the incidences in different organs varying greatly. Also, the incidence of amyloidosis varied between the sexes. Table 8 summarizes data on amyloid deposition. An increase in amyloid deposition was seen in the gallbladder, ileum, salivary gland, and testes of male mice. A decrease in splenic amyloid deposition was noted in both male and female mice. A decrease in amyloid deposition was seen in the heart, kidney, and liver of female mice. The study authors noted that a probable "sparing" effect of amyloid deposition may have contributed to an increased survival among mice receiving 100 or 200 ppm.

2) Neoplastic: Table 9 summarizes the incidence of neoplastic findings in mice in the main group, combining data for those that died, were sacrificed moribund, or were sacrificed at termination. There were no increases in neoplasms at any site except the liver. A significant ($p < 0.01$) increase in the incidence of hepatocytic adenoma in males receiving 100 and 200 ppm was noted. An increased incidence of hepatocytic carcinoma in males receiving 15 and 100 ppm and in females receiving 200 ppm was reported. An increased incidence of hepatocellular tumors (adenoma and carcinoma) was seen in males receiving 15, 100, and 200 ppm and in females receiving 200 ppm. However, the increased incidence of hepatocytic tumors in males receiving 15 ppm was not considered to be treatment-related by the study authors, but rather was considered to be a consequence of increased survival. Age-adjusted tumor incidence was analyzed by the method of Peto combining fatal and incidental tumors. For hepatocellular tumors (adenomas and carcinomas combined), the adjusted rates in males were 16.1, 17.2, 39.0, 52.2, and 32.7%, and in females the rates were 0, 0, 0, 6.1, and 33.6% at dose levels of 0, 5, 15, 100, or 200 ppm, respectively. The increase in males was significant at 100 ppm ($p = 0.003$) but not at 15 ppm ($p = 0.188$) or at 200 ppm ($p = 0.052$). In females, the increase was significant at 200 ppm ($p = 0.001$) but not at 10 ppm ($p = 0.135$). There was a significant dose trend in both males ($p < 0.01$) and females ($p < 0.001$). The authors' interpretation of the hepatocarcinogenic no-effect level of 15 ppm for males was based on a consideration of the greater longevity of male mice receiving 15, 100, and 200 ppm, and of females receiving 200 ppm, and the results of the Peto statistical analysis using

by the authors

TABLE 8. Amyloid Deposition in Mice Fed Cyproconazole for 88 Weeks[a,b]

Organ/Finding	Dietary Level (ppm)									
	Males					Females				
	0	5	15	100	200	0	5	15	100	200
Liver	(100)[c]	(50)	(50)	(50)	(50)	(100)	(50)	(50)	(50)	(50)
	23	4*	12	4*	8	32	19	16	6***	3***
Heart, ventricle	(100)	(50)	(50)	(50)	(50)	(99)	(50)	(50)	(50)	(50)
	21	3	10	5	10	27	8	5*	2***	4**
Testes	(100)	(50)	(50)	(50)	(50)	(0)	(0)	(0)	(0)	(0)
	35	20	22	25	27*	0	0	0	0	0
Gallbladder	(97)	(49)	(49)	(48)	(48)	(99)	(49)	(48)	(49)	(49)
	0	1	0	0	5**	2	2	1	1	0
Ileum	(100)	(50)	(50)	(50)	(50)	(100)	(50)	(50)	(50)	(49)
	44	24	32	28	34**	44	29	30	35**	27
Salivary gland	(100)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
	10	4	13*	11	16**	31	11	14	20	16
Spleen	(100)	(50)	(50)	(50)	(50)	(99)	(50)	(50)	(50)	(50)
	26	7	10	3**	1***	29	13	14	1***	1***
Adrenal gland	(100)	(50)	(47)	(49)	(50)	(100)	(50)	(49)	(50)	(50)
	54	29	27	26	25	44	22	28	18	18
Kidney	(100)	(50)	52 (47)	50 (49)	(50)	(100)	(50)	50 (49)	(50)	(50)
	57	28	31	25	28	54	28	31	28	21

[a]Female mice were treated for 88 weeks, males for 81 weeks.

[b]Fischer's two-tailed Exact test comparing pooled controls vs. the treated groups was used for statistical evaluation of the data.

[c]The numbers in parentheses are the numbers of animals with specific organ examined microscopically.

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

***Significantly different from control value ($p < 0.001$).

TABLE 9. Neoplastic Lesions in Mice Fed Cyproconazole for 88 Weeks[a,b]

Organ/Finding	Dietary Level (ppm)									
	Males					Females				
	0	5	15	100	200	0	5	15	100	200
Liver	(100)[c]	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Hepatocytic Adenoma	6[d]	4	5	12**	12**	0	0	0	2	6**
Hepatocytic Carcinoma	0	0	3*	3*	1	0	0	0	0	7**
Hepatocellular Adenoma/Carcinoma	6[e]	4	8*	15***	13***	0	0	0	2	13***
Lungs	(100)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Pulmonary adenoma	10	3	7	4	2	9	3	5	1	3
Pulmonary carcinoma	3	0	0	1	1	0	0	0	1	0
Mammary gland	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Adenocarcinoma (Type B)	0	0	0	0	0	1	0	2	1	0
Spleen	(100)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Hemangiosarcoma	0	0	0	0	0	2	1	0	1	0
Uterus + Cervix	(0)	(0)	(0)	(0)	(0)	(100)	(50)	(50)	(50)	(50)
Leiomyosarcoma	0	0	0	0	0	7	2	3	3	4
Leiomyona	0	0	0	0	0	3	5	4	4	5
Fallopian tube adenoma	0	0	0	0	0	1	0	0	0	0
Endometrial carcinoma	0	0	0	0	0	2	0	0	0	1

[a]Female mice were treated for 88 weeks, males for 81 weeks.

[b]Includes animals in main study that died, were sacrificed moribund, or were sacrificed at study termination.

[c]The numbers in parentheses are the numbers of animals with specific tissue examined histologically.

[d]Fischer's one-tailed Exact test comparing pooled controls vs. the treated groups was used for statistical evaluation of the data.

[e]Significant trend by the Cochran-Armitage test ($p < 0.001$).

*Significantly different from control incidence ($p < 0.05$).

**Significantly different from control incidence ($p < 0.01$).

***Significantly different from control incidence ($p < 0.001$).

lifetable methodology to compensate for this extended longevity. Non-treatment-related neoplasms that occurred with an incidence of 2% or less in all groups included, in female mice, medullary adenomas, malignant lymphoma of the ileum, pituitary adenomas, osteogenic sarcoma, and skin basal cell tumor. In males, incidental neoplasms included oligodendroglioma, leiomyoma, skin merkel cell tumor, splenic hemangioma, testicular interstitial cell adenoma, urinary bladder papilloma, and hemangiosarcoma of the diaphragm.

D. STUDY AUTHORS' CONCLUSIONS:

The primary treatment-related effects of cyproconazole were observed in the liver, and included both toxic and neoplastic changes. There were increased incidences of combined adenomas and carcinomas in males fed cyproconazole at dietary levels of 15, 100, and 200 ppm, and in females fed 200 ppm. Appropriate statistical analysis (the method of Peto) revealed that the apparent increase in hepatocytic neoplasia seen at 15 ppm was due to increased survival among these mice. Male and female mice receiving 100 and 200 ppm had increased incidences of focal hepatocytic inflammation, single-cell necrosis, and diffuse hepatocytic hypertrophy; male mice were more severely affected. An increased incidence of periacinar hepatocytic vacuolation was seen in females treated with 100 ppm. Other changes that were associated with treatment included a decrease in the amount of testicular germinal epithelium in males receiving 200 ppm, and possibly an increase in skin ulcerations in all treated groups of both sexes, although statistically significantly different only in female mice of the 200-ppm dose group. The increased testicular germinal epithelial deficit corresponded to the increased incidence of flaccid testes in male mice of the 200-ppm dose group. Body weight gains were reduced by more than 10% in males and females fed cyproconazole at dietary levels of 100 and 200 ppm. At these dose levels, a "sparing" effect of amyloid deposition may have contributed to an increased survival among these mice. An increase in the liver weights of both sexes were seen following treatment with 200 ppm for 13 weeks when compared to controls for both the absolute weight and the weight relative to body weight. Increases in liver weights were also seen in females of the 100-ppm group at termination. The increase in liver weights was not a consequence of neoplasia. No effects on other organ weights were seen in either sex receiving 5, 15, 100, or 200 ppm. There was no effect of dosing on mortality, food consumption, or hematology parameters. The no-effect level (NOEL) for toxicity was established at 15 ppm.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was complete and adequate, and the data were well reported. Summary data were supported by individual animal data, and mean values that were validated agreed with the authors' values.

Actual dietary values were generally in agreement with nominal values; a deviation greater than ±20% from the normal limits was encountered on several occasions, especially among the low-level diets. However, the variations were not sufficient in degree or frequency to affect the interpretation of the study. The findings of this study suggest that the liver ~~may be~~ is a target organ for cyproconazole toxicity. The primary treatment-related alterations observed in the livers of male and female mice following oral treatment with cyproconazole (100 and 200 ppm) in the diet included focal hepatocytic inflammation, single-cell necrosis, and diffuse hepatocytic hypertrophy. An increased incidence of centriacinar hepatocytic vacuolation was seen in male mice receiving 200 ppm and in females receiving 100 and 200 ppm. Periacinar hepatocytic vacuolation was noted in females treated at 100 ppm. Male mice were more sensitive to the toxic effects of cyproconazole than female mice. Histological changes in the liver correlated with gross pathological changes (accentuated lobular pattern) and increased organ weight. Liver weights in males were increased by 41% at week 14 and by 34% at termination. Liver weights in females were increased by 44% at week 14 and 52% at termination. The incidence of amyloid deposition varied greatly among organs and between male and female mice. Increased incidences of amyloid deposition were seen in the gallbladder, ileum, salivary gland, and testes of male mice. Decreased incidences of amyloid deposition were observed in the spleens of both sexes and in the heart, kidney, and liver of females. Although the study authors concluded that a "sparing" effect of amyloid deposition may have contributed to increased survival among the high-dose male and female groups, the data are insufficient to support the conclusion. No data were provided that showed a correlation between amyloid deposition and increased survival among mice. In addition, the changes in amyloid deposition were minimally to moderately severe and included increases as well as decreases in incidence. Increases in amyloid deposition are usually associated with cellular infiltration and degeneration of organs. The results of this study also indicate that cyproconazole is a possible carcinogen in male CD-1 mice. Increased incidences of combined liver adenomas and carcinomas were detected in females receiving 200 ppm, and in males receiving 15, 100, and 200 ppm. ~~Appropriate statistical analysis by the method of Peto to determine age-adjusted tumor incidence indicated that the apparent increase in hepatocellular tumors seen at the 15-ppm dose level was due to~~

~~increased survival among these mice. The maximum tolerated dose is 100 ppm based on effects on body weight.~~

We agree with the study authors' assessment of a NOEL of 15 ppm for systemic toxicity.

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EPA No.: 68D80056
DYNAMAC No.: 244-B
TASK No.: 2-44B
April 10, 1990

CASWELL FILE

DATA EVALUATION RECORD

SAN 619F (Cyproconazole)

Chronic Toxicity/Oncogenicity Feeding Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: April 10, 1990

(44)

EPA No.: 68D80056
DYNAMAC No.: 244-B
TASK No.: 2-44B
April 10, 1990

DATA EVALUATION RECORD

SAN 615 F (Cyproconazole)

Chronic Toxicity/Oncogenicity Feeding Study in Rats

REVIEWED BY:

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Date: 4/18/90

DATA EVALUATION RECORD

GUIDELINE §85-5

STUDY TYPE: Chronic toxicity/oncogenicity feeding study in rats.

MRID NUMBER: 411647-01.

TEST MATERIAL: SAN 619F.

SYNONYM: Cyproconazole.

STUDY NUMBER: 357-R.

SPONSOR: Sandoz Crop Protection Corporation.

TESTING FACILITY: Sandoz Ltd. Agro Development, Toxicological Department Basle/Switzerland.

TITLE OF REPORT: San 619F Chronic Toxicity/Oncogenicity Feeding Study in Rats.

AUTHORS: Warren, S.F.P., Carpy, S., and Müller, F.

REPORT ISSUED: April 22, 1988.

CONCLUSIONS:

Under the conditions of the study, SAN 619F was not carcinogenic when fed to KFM-Wistar rats for up to 118 (males)/121 (female) weeks at dietary levels of 0, 20, 50, or 350 ppm (corresponding to an intake of 1.0, 2.2, and 15.6 mg/kg/day for males and 0, 1.2, 2.7, and 21.8 mg/kg/day for females). At the 350-ppm dose, mean body weights were significantly depressed in females throughout most of the study, although the differences were generally less than 10% below control values. In males, slightly (<6% below control value) depressed body weights were observed in the high dose compared to control. Mean weight gains from initiation to week 13 or 79 were depressed 6 to 8% in high-dose males and 10 to 15% in high-dose females. Food consumption was not affected. An increase in the incidence of fatty change was observed in the liver of males receiving 350 ppm. Hepatocellular hypertrophy was observed in females receiving 350 ppm at the 78-week sacrifice (4/10 rats), but the lesion was not observed in the main group of animals or at the 52-week sacrifice. Liver-to-body weight ratios were significantly increased in females receiving 350 ppm at both 12 and 18 months and at terminal sacrifice. Serum alanine aminotransferase activity was significantly increased at week 118 and aspartic aminotransferase was significantly increased at 78 weeks in males at the 350 ppm dose level. Cholesterol levels were increased in mid- and high-dose females at 78, 105, and 121 weeks, although the increase was significant only at 105 weeks (not dose-related). No effects were observed on any parameter at the low dose level.

Based on the lack of: 1) any biologically significant body weight decrement; 2) any significant histopathological correlate accompanying the increased relative liver weight in the high-dose females; 3) any increase in the liver enzyme activities in the female; 4) any consistent change in the liver enzyme activities in the high-dose males, suggests that the dose levels chosen were not adequate to determine the carcinogenic potential of the test material.

Based on the decreased body weights in high-dose females and the increased incidences of fatty infiltration of the liver in high-dose males, the LOEL for systemic toxicity is 350 ppm and the NOEL is 50 ppm.

Classification: Core Supplementary under guideline 83-2, Oncogenicity Study.

Core Minimum under guideline 83-1, Chronic Toxicity Study.

A. MATERIALS:

1. Test Compound: SAN 619F; description: light brown powder; batch No.: 8507; purity: 95.6 ± 1%.

2. Test Animals: Species: Albino rat; strain: KFM Wistar (of HAN Wistar origin); age: 7 weeks at initiation; weight: males--172 g to 229 g; females--126 to 175 g; source: KFM Breeders, Switzerland.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimated to laboratory conditions for 14 days, and were assigned randomly by sex to the following test groups using a computer-generated randomization procedure:

Test group	Dose in diet (ppm)	Number of Animals: Main study (24 months) Males	Main study (24 months) Females	Interim sacrifice (12 months) Males	Interim sacrifice (12 months) Females	Interim sacrifice (18 months) Males	Interim sacrifice (18 months) Females
1 (K) Control	0	50	50	10	10	10	10
2 (A) Low (LDT)	20	50	50	10	10	10	10
3 (B) Mid (MDT)	50	50	50	10	10	10	10
4 (C) High (HDT)	350	50	50	10	10	10	10

Rats were housed individually in a room with temperature and humidity set at 23°C and 50%, respectively, with a 12-hour light/dark cycle. Animals received ear punchmarks, allowing individual identification, plus color-coded and individually numbered cage labels.

2. Diet Preparation: Premixes were prepared by mixing SAN 619F with powdered diet (Kliba No. 21-343-4) for 1 hour to produce a premix with a concentration of 10 mg SAN 619F per gram (1%). This diet premix was renewed at least monthly. Final diets were prepared weekly by mixing diet premix with additional powdered diet to achieve the appropriate concentrations. The stability, homogeneity, and concentration of the premixes and diets were analyzed before the start of the study and at monthly intervals thereafter.

Results:

The analyzed concentrations of test material in the diets were reported as generally acceptable and within 10% of nominal concentrations. All diets were higher (22 to 50%) than nominal concentrations at 28 weeks. Deviations from nominal were greater than 15% at seven, six, and two intervals of analysis at nominal levels of 20, 50, and 350 ppm, respectively. Mean concentrations for the entire study, however, were within 5% of the nominal at all dose levels (Table 1). Data on homogeneity and stability were not provided but reference is made to indicate that both are documented elsewhere.

TABLE 1. Mean Concentrations of SAN 619F in Formulated Rodent Diet

Treatment Group	Nominal (ppm)	n (weeks)	Mean Analyzed Concentration (% of Nominal ± S.D.)
1% Premix	10,000	31	102.1 ± 6.35
A	20	31	105.3 ± 10.48
B	50	31	101.5 ± 13.47
C	350	31	99.1 ± 7.79

3. Food and Water Consumption: Animals received Kliba No. 21-343-4 powdered diet and tap water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data: Levene's test was used to analyze homogeneity of variances. One-way analysis of variance (ANOVA) was used for data with homogeneous variance, and if significant differences between groups were found, pairwise comparison with controls using Dunnett's test was performed. Nonparametric data were analyzed by the Kruskal-Wallis test followed by multiple comparisons with the Dunn-Bonferroni test; the Mann-Whitney U-test was used if there was only one treatment group.

5. Quality Assurance: A quality assurance statement was signed and dated April 22, 1988.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of morbidity and mortality. Detailed examinations and

palpation of masses were conducted biweekly. Cages were inspected for traces of blood or abnormal feces/urine.

Results: No compound-related signs of toxicity were seen. A small number of rats (nine in all groups) were observed with skin ulceration, which was considered to be due to bacterial infection. They were treated with an anti-bacterial aerosol spray.

Survival was not affected by dosing. Table 2 summarizes data for mortality and percent survival in the main groups. Animals in the satellite groups that died before the 12- and 18-month interim sacrifices were replaced by animals from the main groups.

2. Body Weight: Body weights were recorded weekly for the first 13 weeks of treatment; at weeks 15, 17, 19, and 21; and then weekly thereafter.

Results: Tables 3 and 4 present mean body weight data and weight gain data, respectively, for selected intervals during the study. Mean body weights in males receiving 20 and 50 ppm tended to be slightly increased when compared to controls throughout the study. Mean body weights in high-dose males were 4 to 6% lower than controls, but the differences were not consistently significant. In females receiving 350 ppm, the mean body weights were consistently lower ($p < 0.05$) than controls from weeks 1 to 104 although the increase did not exceed 10% except at week 104. Mean weight gains during the first 2 weeks of the study were 12 and 17% lower in high-dose males and females than in their respective controls, suggesting a palatability problem. However, this was not supported by the data on food consumption (see below). For the first 79 weeks, the respective gains were 8 and 15% lower than control gains in males and females receiving 350 ppm (Table 4); however, the mean weekly gains in both sexes were only 0.5 g lower in the high dose than in controls.

3. Food Consumption and Compound Intake: Consumption was measured, and mean daily dietary consumption was calculated on a weekly basis, up to 13 weeks of treatment. Consumption was then determined for weeks 15, 17, 19, 21 and weekly thereafter. Diet wastage was estimated and corrected for. Efficiency and compound intake were calculated from the consumption and body weight gain data.

Results: Food consumption, in general, was similar in dosed and control groups. It was increased (6%) in high-dose females but not males during the first 13 weeks of the study as compared to controls; however, the study authors considered that the increased consumption was caused by

TABLE 2. Cumulative Mortality and Percent Survival in Rats Fed SAN 619F for 121 Weeks[a]

Dose Group (ppm)	Mortality (Percent Survival) at Week: 52	80	92	104	Termination[b]
			Males		
0	2 (96)	11 (78)	12 (76)	20 (60)	36 (28)
20	2 (96)	9 (82)	14 (72)	25 (50)	34 (32)
50	3 (94)	12 (76)	19 (62)	22 (56)	30 (40)
350	0 (100)	4 (92)	12 (76)	17 (66)	28 (44)
			Females		
0	2 (96)	10 (80)	15 (70)	21 (58)	32 (36)
20	2 (96)	8 (84)	11 (78)	16 (68)	31 (38)
50	1 (98)	7 (86)	11 (78)	13 (74)	29 (42)
350	1 (98)	5 (90)	7 (86)	18 (64)	33 (34)

[a]Mortality and percent survival were based on 50 rats/sex/dose of the main group. An additional 20 rats/sex/dose (10/sex/dose at 52 weeks and 10/sex/dose at 78 weeks) survived until their respective scheduled-sacrifice dates and are not included in this table.

[b]Males were terminated at week 118 and females at week 121.

TABLE 3. Mean Body Weights at Selected Intervals for Rats Fed SAN 619F for 121 Weeks

Dietary Level (ppm)	Mean Body Weight (g ± S.D.) at Study Weeks: 0	13	26	52	78	104	118 (males) 121 (females)
				Males			
0	196 ± 11	429 ± 36	480 ± 42	573 ± 58	613 ± 67	605 ± 83	586 ± 133
20	198 ± 11	440 ± 34	493 ± 35	587 ± 48	621 ± 56	628 ± 70	571 ± 72
50	195 ± 10	437 ± 35	491 ± 41	584 ± 62	636 ± 64	642 ± 81	588 ± 70
350	195 ± 13	417 ± 41	461 ± 43*	547 ± 56*	583 ± 68	574 ± 85	551 ± 42
				Females			
0	144 ± 9	246 ± 22	269 ± 25	330 ± 41	379 ± 59	407 ± 60	397 ± 75
20	145 ± 7	246 ± 19	267 ± 22	327 ± 44	368 ± 51	398 ± 67	397 ± 66
50	146 ± 8	249 ± 20	270 ± 22	328 ± 34	376 ± 47	406 ± 72	410 ± 63
350	145 ± 7	237 ± 17*	254 ± 20**	298 ± 34**	343 ± 51**	353 ± 51**	362 ± 68

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

TABLE 4. Mean Weight Gains (g ± S.D.) at Selected Intervals for Rats Fed SAN 619F

Dietary Level (ppm)	Weight Gain (g/rat/week) During Weeks: 0-2	0-13	0-79
		Males	
0	38.8 ± 4.6	18.1 ± 2.3	5.9 ± 0.8
20	40.0 ± 3.8	18.7 ± 2.2	5.9 ± 0.7
50	39.6 ± 4.9	18.6 ± 2.3	6.1 ± 0.8
350	34.3 ± 6.5**	17.1 ± 2.7	5.4 ± 0.9
		Females	
0	17.3 ± 2.9	7.9 ± 1.3	3.3 ± 0.6
20	17.1 ± 2.7	7.8 ± 1.2	3.1 ± 0.7
50	17.5 ± 3.2	8.0 ± 1.2	3.1 ± 0.7
350	14.4 ± 2.7**	7.1 ± 1.0**	2.8 ± 0.6**

**Significantly different from control value ($p < 0.01$).

food scattering. Food consumption for weeks 1 to 79 was similar in control (122 ± 11 g/rat/week) and high-dose females (123 ± 11 g/rat/week). A slight decrease in food efficiency was observed in high-dose males and females during the first 13 weeks. The food conversion ratio (mean food consumption divided by mean weight gain) was 9.9 in high-dose males as compared to 9.4 for controls, and in high-dose females the ratio was 19.0 as compared to 16.1 for controls. The calculated intakes of SAN 619F were 1.01, 2.22, and 15.59 mg/kg/day for males at dietary levels of 20, 50, or 350 ppm and 1.24, 2.73, and 21.76 mg/kg/day for females at the same doses.

4. Ophthalmological Examinations: Ophthalmologic examinations were performed on one occasion only, during weeks 98 and 99, on all surviving rats of the control and high-dose groups, respectively.

   Results: No treatment-related ophthalmic findings were noted.

5. Hematology and Clinical Chemistry: Blood was collected by superficial venesection of the sublingual vein at weeks 14, 26, 52, 78, 105, and termination for hematology and clinical analysis from 10 male and 10 female animals of each dose group. Blood was collected from the same animals for the first three intervals (those scheduled for the 52-week sacrifice), from the animals sacrificed at 78 weeks, and from 10/sex/group at termination. The CHECKED (X) parameters were examined:

   a. Hematology:

X	Hematocrit (HCT)†	X	Leukocyte differential count
X	Hemoglobin (HGB)†	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)†	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)†	X	Mean corpuscular volume (MCV)
X	Platelet count†		Coagulation: thromboplastin time (PT)
X	Reticulocyte count (RETIC)		
	Red cell morphology		

   Results: No effects of toxicological importance were observed on any hematologic parameter. Reticulocyte counts were slightly, and nonsignificantly, increased in high-dose females at 14 weeks but not at other intervals. Total WBC were slightly decreased in high-dose males at 14 weeks.

†Recommended by Subdivision F (November 1984) Guidelines.

b. Clinical Chemistry

	Electrolytes		Other
X	Calcium†	X	Albumin†
X	Chloride†		Albumin/globulin ratio
	Magnesium†	X	Blood creatinine†
X	Phosphorus†	X	Blood urea nitrogen†
X	Potassium†	X	Cholesterol†
X	Sodium†	X	Globulins
		X	Glucose†
	Enzymes	X	Total bilirubin†
X	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase	X	Total protein†
X	Creatine phosphokinase†[a] (CPK)	X	Triglycerides
	Lactic acid dehydrogenase	X	Hemolytic score
X	Serum alanine aminotransferase (ALAT)†		
X	Serum aspartate aminotransferase (ASAT)†		
X	Gamma glutamyltransferase[a] (GGT)		

Results: Total bilirubin levels were reported to be lower in high-dose males and females than in controls throughout the study. The decreases did not reach a level of significance and were most obvious at weeks 14, 26, and 52 in males and weeks 14 and 26 in females. Although levels were slightly decreased in high-dose rats of both sexes at weeks 78, 105, and at termination, there were no dose-related trends. These changes were not considered to be of toxicologic importance or to be related to dosing.

Cholesterol levels were significantly ($p < 0.05$) increased compared to controls at 105 weeks in mid- and high-dose females but this increase was not dose related. Similar increases were seen at 78 and 121 weeks, but the increases did not reach a level of significance. There were no effects on cholesterol levels in males. It is to be noted that increased cholesterol values have been observed in other studies on the test material. Triglyceride levels tended to be decreased in high-dose females except at week 121; slight decreases were also seen in mid- and low-dose females at 14, 26, and 52 weeks, but there was no clear dose-related trend; a level of significance was not

†Recommended by Subdivision F (October 1982) Guidelines.

[a]CPK and GGT were not analyzed after week 78. In addition, serum corticosterone levels were measured at 52 weeks, and liver samples from rats sacrificed at 52 weeks were frozen and analyzed for glycogen content and glucose-6-phosphatase and fructose-1,6-di-phosphatase activities.

reached. Triglyceride levels were somewhat decreased in males receiving 350 ppm at weeks 14, 26, and 52, but the decrease was only significant ($p < 0.05$) at 26 weeks. Table 5 presents mean data for cholesterol and triglycerides for females.

Table 6 presents mean data for ASAT, ALAT, and GGT in male rats. At week 78, ASAT, ALAT, and GGT activities were increased in males receiving 350 ppm, but the increase was significant only for ASAT ($p \leq 0.01$). The increases in the mean activity of these enzymes in high-dose males at week 78 were a reflection of the high levels of the activities of all enzymes detected in 3 of the 10 animals. These same three males were not sampled at either earlier or later bleeding intervals. Increases were also observed in mean ASAT and mean ALAT activity in high-dose males at 118 weeks, but the increase was significant for only ALAT.

Urea levels in females receiving 350 ppm were consistently 8 to 10% higher than in controls, but these changes were considered marginal and no effects were seen in males. Total protein and globulin levels were slightly increased in high-dose females at 78, 105, and 121 weeks. The increases were significant at week 78 ($p < 0.05$) and 121 ($p < 0.01$). No other clinical chemistry finding could be correlated with dosing.

Analysis of livers of rats sacrificed after 52 weeks for glycogen content, glucose-6-phosphatase, and fructose-1,6-diphosphatase activities did not indicate any effects of dosing; serum levels of corticosterone were unaffected at the same interval.

6. Urinalysis: Urine was collected from 10 fasted rats per sex per group during weeks 14, 26, 52, 78, and 105, and prior to termination. The CHECKED (X) parameters were examined:

X	Appearance†	X	Glucose†
X	Volume†	X	Ketones
X	Specific Gravity†	X	Bilirubin†
X	pH	X	Blood†
X	Sediment (microscopic)†		Nitrate
X	Protein†	X	Urobilinogen

Results: No compound-related effects on urinary parameters were observed.

†Recommended by Subdivision F (October 1982) Guidelines.

TABLE 5. Mean Cholesterol and Triglyceride Levels in Female Rats Fed SAN 619F

Dietary Level (ppm)	Mean Value (± S.D.) at Week: 14	26	52	78	105	121
			Cholesterol (mmol/L)			
0	1.03 ± 0.30	1.17 ± 0.39	2.15 ± 0.93	1.64 ± 0.49	1.66 ± 0.34	1.83 ± 0.40
20	0.81 ± 0.43	0.97 ± 0.58	1.98 ± 0.51	1.65 ± 0.47	1.99 ± 0.43	2.38 ± 1.07
50	0.98 ± 0.39	1.21 ± 0.40	1.96 ± 0.64	2.19 ± 1.68	2.21 ± 0.37*	2.47 ± 0.68
350	0.92 ± 0.38	1.07 ± 0.43	1.78 ± 0.29	2.21 ± 0.42	2.14 ± 0.54*	2.85 ± 0.94
			Triglycerides (mmol/L)			
0	0.65 ± 0.27	0.70 ± 0.31	1.16 ± 0.86	1.01 ± 0.58	1.29 ± 0.58	1.11 ± 0.59
20	0.47 ± 0.13	0.48 ± 0.13	0.73 ± 0.30	0.84 ± 0.41	1.41 ± 0.87	1.54 ± 0.99
50	0.55 ± 0.19	0.58 ± 0.14	0.81 ± 0.51	1.44 ± 2.16[a]	1.21 ± 0.56	1.32 ± 0.58
350	0.51 ± 0.17	0.51 ± 0.14	0.70 ± 0.36	0.64 ± 0.16	0.93 ± 0.23	1.58 ± 0.77

[a]The mean calculated excluding one outlier value is 0.85 mmol/L.

*Significantly different from control value ($p < 0.05$).

TABLE 6. Mean Activity of Aspartic Aminotransferase, Alanine Aminotransferase, and Gamma Glutamyl Transferase in Male Rats Fed SAN 619F

Parameter/ Dietary Level (ppm)	Mean Activity (U/L ± S.D.) at Week: 14	26	52	78	105	118
ASAT						
0	41.7 ± 7.3	42.8 ± 15.7	39.8 ± 7.4	36.0 ± 7.6	39.5 ± 8.9	49.1 ± 10.6
20	43.8 ± 4.9	42.1 ± 14.0	33.8 ± 2.8	39.8 ± 10.0	43.8 ± 15.8	51.0 ± 25.6
50	43.9 ± 3.3	42.5 ± 7.7	43.2 ± 12.9	44.2 ± 15.5	43.4 ± 7.9	48.6 ± 14.1
350	42.9 ± 4.4	39.4 ± 5.8	42.2 ± 7.9	62.5 ± 34.0**	42.6 ± 13.1	63.5 ± 30.0
ALAT						
0	20.6 ± 3.5	24.7 ± 13.9	19.5 ± 3.3	19.5 ± 3.8	23.2 ± 10.6	21.0 ± 6.0
20	21.6 ± 3.9	26.9 ± 25.0	18.4 ± 5.9	20.9 ± 4.7	26.8 ± 14.7	37.7 ± 43.5[a]
50	22.5 ± 3.6	23.6 ± 8.3	22.9 ± 11.3	26.6 ± 9.6	19.5 ± 5.0	21.0 ± 6.8
350	23.2 ± 4.0	23.0 ± 3.8	22.0 ± 5.6	58.1 ± 71.6	24.0 ± 13.5	31.8 ± 10.2**
GGT						
0	0.7 ± 1.0[b]	0.7 ± 0.7	0.1 ± 0.2	0.9 ± 2.0[c]		
20	0.5 ± 0.6	0.8 ± 0.7	0.2 ± 0.2	0.3 ± 0.3		
50	0.6 ± 0.6	0.9 ± 0.7	0.3 ± 0.4	0.3 ± 0.3		
350	0.7 ± 0.6	1.1 ± 0.3	0.3 ± 0.5	4.1 ± 8.6[d]		

[a]If an outlier value of 151 is not included, the mean value is 23.5.

[b]If an outlier value of 3.2 is not included, the mean value is 0.4 ± 0.4.

[c]If an outlier of 6.4 is not included, the mean value is 0.3 ± 0.5.

[d]If outliers of 3.4, 27.1, and 9.4 are not included, the mean value is 0.1 ± 0.3.

**Significantly different from control value ($p < 0.01$).

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

	Digestive System		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta†	XX	Brain†
X	Salivary glands†	XX	Heart†	X	Peripheral nerve (sciatic nerve)†
X	Esophagus†	X	Bone marrow†		
X	Stomach†	X	Lymph nodes† (cervical & mesenteric)	X	Spinal cord (3 levels)
X	Duodenum†	XX	Spleen†	XX	Pituitary†
X	Jejunum†	X	Thymus†	X	Eyes (optic nerve)†
X	Ileum†				
X	Cecum†				
X	Colon†				
X	Rectum		Urogenital		Glandular
XX	Liver†	XX	Kidneys†	XX	Adrenals†
	Gallbladder†	X	Urinary bladder†		Lacrimal gland
X	Pancreas†	XX	Testes†	X	Mammary gland†
		X	Epididymes	X	Thyroids†
		X	Prostate	X	Parathyroids†
		X	Seminal vesicle		Harderian glands
	Respiratory	XX	Ovaries		
X	Trachea†	X	Uterus†		
X	Lung†				
					Other
				X	Bone (sternum and femur)†
				X	Skeletal muscle†
				X	Skin
				X	All gross lesions and masses

Results:

a. Organ Weights: Table 7 presents data on liver weights. The liver-to-body weight ratios in high-dose females were significantly increased ($p < 0.01$) when compared to controls at the 52-, 78-, and 121-week sacrifices. There were only slight increases in the absolute liver weights in these females at 78 and 121 weeks. The mean body weights at necropsy were 20, 15, and 16% lower than controls in high-dose females at 52, 78, and 121 weeks. There were no corresponding effects on liver

†Recommended by Subdivision F (October 1982) Guidelines.

TABLE 7. Mean Liver Weights (g ± S.D.) and Liver-to-Body Weight Ratios (% S.D.) in Rats Fed SAN 619F

Dietary Level (ppm)	Week 52 (g)	Week 52 (%)	Week 78 (g)	Week 78 (%)	Termination[a] (g)	Termination[a] (%)
			Males			
0	14.44 ± 1.59	2.51 ± 0.23	14.94 ± 2.15	2.52 ± 0.23	17.0 ± 3.3	3.26 ± 0.92
20	14.54 ± 1.46	2.51 ± 0.15	15.68 ± 2.21	2.58 ± 0.30	16.3 ± 2.3	3.02 ± 0.43
50	13.83 ± 1.86	2.46 ± 0.25	16.23 ± 2.51	2.56 ± 0.30	17.6 ± 2.8	3.06 ± 0.39
350	13.90 ± 1.30	2.61 ± 0.20	16.24 ± 2.45	2.77 ± 0.34	18.2 ± 3.6	3.55 ± 0.60
			Females			
0	8.71 ± 1.77	2.62 ± 0.18	8.58 ± 0.84	2.46 ± 0.31	13.3 ± 3.7	3.43 ± 0.78
20	8.06 ± 0.99	2.70 ± 0.23	8.81 ± 1.10	2.50 ± 0.22	13.0 ± 2.8	3.58 ± 1.04
50	8.86 ± 1.12	2.82 ± 0.34	9.86 ± 2.17	2.69 ± 0.58	13.1 ± 2.6	3.44 ± 0.79
350	8.13 ± 1.01	3.06 ± 0.25**	9.55 ± 0.80	3.19 ± 0.23**	13.9 ± 2.7	4.31 ± 0.60**

[a]Males were sacrificed at 118 weeks and females at 121 weeks. Values at termination are means for 14, 16, 20, and 22 (males) and 18, 19, 21, and 17 (females) at dietary levels of 0, 20, 50, and 350 ppm, respectively.

**Significantly different from control values ($p < 0.01$).

weights in males, and necropsy body weights did not differ significantly in the control and high-dose groups.

Kidney-to-body weight ratios in high-dose females were slightly increased at 78 weeks ($p < 0.05$) and 121 weeks ($p < 0.01$), but there were no effects on absolute kidney weights. Moderate but significant increases in relative heart and brain weight were also observed in females receiving 350 ppm ($p < 0.05$ for brain at 78 and 121 weeks and for heart at 52 weeks, and $p < 0.01$ for brain at 52 weeks and for heart at 78 and 121 weeks); no effects on absolute weights of heart and brain were observed, nor were corresponding effects seen in males.

b. Gross Pathology: It was reported that the recorded gross findings were those commonly found in rats of this strain and that their incidence was similar in treated and control groups. A summary tabulation of gross findings was not presented. A scan of the individual pathology sheets (for high-dose animals) did not reveal any unusual findings.

c. Microscopic Pathology:

1) Nonneoplastic: An increase of fatty changes of the liver (hepatocyte vacuolization) was observed in high-dose males. Table 8 summarizes the incidence and severity of fatty changes in the liver at the 52- and 78-week sacrifices and for animals in the main study (includes deaths, moribund sacrifices, and terminal-sacrifice rats). Both the incidence and severity of the changes were increased. There were significant positive trends ($p = 0.0026$ at 52 weeks and $p < 0.0005$ at 78 weeks and in the main study group). The incidence was not increased in dosed females; in fact, it was decreased in the high-dose group (main group). Hepatocellular hypertrophy (mild) was observed in four females receiving 350 ppm at 78 weeks; it was not seen in female rats in the main groups or in those sacrificed at week 52. One mid-dose male and one high-dose male (main group) displayed this lesion also. The authors considered that all other nonneoplastic lesions noted in the study were incidental and commonly found in rats of this age and strain. Table 9 summarizes frequent non-neoplastic findings in the main study. A similar pattern of nonneoplastic findings was seen at the 52- and 78-week sacrifices.

2) Neoplastic: Table 10 presents selected neoplastic lesions in the main groups. There were no compound- or dose-related increases in neoplasms.

TABLE 8. Incidence and Severity of Fatty Changes in the Livers of Rats Fed SAN 619F

Incidence/ Grade[a]	Dietary Level (ppm)							
	Males				Females			
	0	20	50	350	0	20	50	350
52-Week Sacrifice (10/sex/group)								
Total incidence	4	5	7	10	1	0	0	1
Minimal/slight	4	5	7	4	1	0	0	1
Moderate	0	0	0	6	0	0	0	0
78-Week Sacrifice (10/sex/group)								
Total incidence	3	1	4	10	0	0	0	0
Slight	3	1	4	5	0	0	0	0
Moderate	0	0	0	5	0	0	0	0
Main Study (50/sex/group)								
Total incidence	23	19	29	38	23	15	15	10
Minimum/slight	9	5	19	18	13	5	12	3
Moderate/marked	14	14	9	20	4	1	0	3
Massive	0	0	1	0	0	0	0	0

[a]The lesions were graded on the basis of 1 to 5:
1 = minimum
2 = slight
3 = moderate
4 = marked
5 = massive.

TABLE 9. Selected Nonneoplastic Findings in Rats Fed SAN 619F for up to 121 Weeks[a]

Organ/ Finding	Dietary Level (ppm)							
	Males				Females			
	0	20	50	350	0	20	50	350
Adrenal	(50)[b]	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Altered cell focus	15	23	26	28	15	21	17	16
Cystic cortical degeneration	0	2	2	1	31	33	37	39
Heart	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Necrosis/fibrosis	35	37	39	35	23	28	31	37
Kidneys	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Chronic nephropathy	34	34	36	38	15	13	8	25
Tubular dilatation	13	9	10	7	25	14	23	17
Tubular pigment	1	3	7	7	8	5	5	8
Mineralization	3	2	1	3	33	34	32	34
Lymphoid infiltration	40	41	31	36	12	18	16	24
Liver	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(50)
Fatty change	23	19	29	38	23	15	15	10
Hyperplastic nodule	3	3	0	2	3	1	1	2
Bile duct proliferation	22	23	19	26	27	35	32	38
Sinusoidal cell pigmentation	1	1	4	5	5	7	6	13
Vacuolated focus	14	18	19	16	2	9	9	9
Lungs	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(50)
Lipoproteinosis	24	21	19	26	6	14	13	13
Thyroid	(50)	(48)	(49)	(50)	(50)	(50)	(49)	(50)
C-cell hyperplasia	4	0	3	7	5	8	5	6
Lymph nodes (Mesenteric)	(47)	(48)	(48)	(49)	(48)	(49)	(47)	(49)
Pigmented macrophages	21	31	33	39	30	34	37	40
Pancreas	(49)	(49)	(50)	(50)	(50)	(50)	(50)	(49)
Islet cell hyperplasia	16	9	21	20	8	7	8	4

[a]Includes animals in the main study that died or were sacrificed moribund as well as those sacrificed at study termination (week 118 for males and week 121 for females).

[b]The numbers in parentheses represent the number of rats with the specific tissue/organ examined microscopically.

TABLE 10. Representative Neoplasms in Rats Fed SAN 691F for up to 121 Weeks[a]

Organ/ Finding	Dietary Level (ppm)							
	Males				Females			
	0	20	50	350	0	20	50	350
Adrenal	(48)	(50)	(50)	(50)	(48)	(49)	(47)	(49)
Medullary tumor (B)	0	1	2	1	0	2	1	1
Hemolymphoreticular	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Malignant lymphoma	2	1	1	0	2	4	2	2
Fibrous histiocytoma	1	3	1	1	1	1	0	1
Liver	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Hepatocellular adenoma	1	0	1	1	0	0	1	1
Hepatocellular carcinoma	0	0	1	1	0	1	0	0
Mammary glands					(49)	(50)	(49)	(49)
Fibroadenoma					17	23	18	9
Adenocarcinoma					4	7	5	5
Adenoma					0	0	0	2
Ovaries					(50)	(50)	(50)	(50)
Theca cell tumor					0	3	3	1
Pancreas	(49)	(49)	(50)	(50)	(50)	(50)	(50)	(49)
Islet cell adenoma	7	7	8	0	9	3	3	0
Pituitary	(50)	(49)	(50)	(49)	(50)	(50)	(50)	(50)
Adenoma	25	29	29	31	41	39	45	47
Thyroid glands	(50)	(48)	(50)	(49)	(50)	(50)	(49)	(50)
Follicular adenoma	0	2	4	1	1	1	1	2
C-cell adenoma	4	2	3	2	3	8	4	3
Lymph node (mesenteric)	(47)	(48)	(48)	(49)	(48)	(49)	(47)	(49)
Hemangioma	5	0	1	5	0	1	1	0
Skin	(50)	(50)	(49)	(49)	(49)	(50)	(49)	(50)
Squamous carcinoma	0	1	2	1	0	0	0	0
Squamous papilloma	0	3	1	1	0	0	0	1
Fibroma	0	3	1	1	0	1	0	0

[a]Includes all rats in the main groups. Incidental neoplasms (2% in any group) have not been included for all tissues.

The incidence of neoplasms was comparable to that of concurrent controls at the 52- and 78-week sacrifices. One liver adenoma was observed in a high-dose male at 12 months; no other liver neoplasms were seen at interim sacrifices.

D. STUDY AUTHORS' CONCLUSIONS:

The authors concluded that SAN 619F was not carcinogenic in KFM-Wistar rats under the conditions of the study. The study clearly meets "published" MTD requirements, since a 6 and 10% retardation in body weight gain was seen at 13 weeks in males and females, respectively, receiving 350 ppm.

The principal effects seen were confined to the liver and included increased incidences of fatty changes and hepatocellular hypertrophy. The authors considered these changes to be a consequence of induction of hepatic enzymes and to be of limited toxicological significance. Increased liver weights, increases in the activities of serum gamma glutamyl transferase, ASAT, and ALAT, and possibly a decrease in bilirubin levels reflect these changes. There was no evidence of an effect of SAN 619F on the endocrine system. Increases in kidney- and heart-to-body weight ratios in high-dose females did not correlate with histologic findings, nor were they found in males; these changes are probably related to body weight decreases and are of doubtful toxicologic importance. The reduced triglyceride levels in dosed females were not considered of toxicologic importance, and the histologic findings (except for the liver) were considered normal for the strain and age of the rats. The NOEL was 20 ppm, which corresponds to a daily intake of 1 mg/kg/day.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was complete (except as discussed below), and the reporting was adequate. It is noted that summary tabulations of gross pathology findings were not provided, and the final (necropsy) body weight of each animal was not recorded on the pathology sheets. Summary tables of all other parameters were accurate (except as noted below) and were supported by individual animal data.

Discrepancies: In Appendix 3, page 278 (Listing of Animal by Week of Death) - control male No. 68 died at week 109 and 111; in Individual Symptomology Table, No. 68 is listed as dying at week 111 (page 288). Animal No. 469 is listed twice; once as a high-dose male (page 279 lists terminal sacrifice for this animal), and once as a high-dose female (78-weeks interim sacrifice on page 277, probably a typo-should be No. 496); animal No. 496 is not listed in the

Listings of Animal by Week of Death Table, but is listed as a high-dose female sacrificed at the 78-week sacrifice.

On page 27 of the study report, regarding hepatocellular hypertrophy, it is stated that male rats of the high dose displayed this lesion after 78 weeks, but not after 121 weeks. The males did not receive treatment for 121 weeks, but were sacrificed at 118 weeks. High-dose females displayed this lesion at 78 weeks.

We agree with the study authors' assessment that SAN 691F was not oncogenic under the conditions of the study. Neoplasms were not increased at any site, and the incidence of some neoplasms in high-dose groups was lower than in controls (mammary fibroadenomas in females and islet cell adenomas in both sexes). Hepatocellular adenomas and carcinomas were not increased compared to controls.

However, we do not agree with the study authors conclusions that a satisfactory maximum tolerated dose was achieved. In a 90-day feeding study in the same rat strain there was no effect on weight gain at 320 ppm. Since no definitive effects were observed in that study other than increased relative liver weight at the high-dose (320 ppm) with 1/15 mid dose males and 6/15 high-dose displaying vacuolated hepatocytes, it is suggested that the study may not have been rigorous enough to adequately predict dose levels for a chronic study.

In the chronic study, the decrease in body weight in high-dose animals compared to controls was not of great magnitude throughout most of the study. Body weight gain over the first 13 weeks was comparable to control (222 g for high-dose males versus 235 for controls and 92 g for high dose females versus 103 g for controls) although the decrement in females showed statistical significance ($p < 0.01$).

The reviewers did not consider the changes in clinical chemistry parameters that were observed and reported to be of toxicologic importance, primarily because of lack of correlations with histologic findings or organ weight changes. Increases in serum cholesterol were observed in females at weeks 78, 105, and 121 (significant only at week 78 in mid- and high doses), but no changes were seen in dosed males. However, the increase in the incidence and severity of fatty liver changes were seen in the males but not the females receiving 350 ppm. Increases in ASAT ($p < 0.05$, week 78) and ALAT ($p < 0.01$, week 118) in males were not accompanied by significant liver weight changes. In females, there were no definitive changes in serum liver enzymes that correlated with observed liver weight changes. Interpretation of clinical chemistry data was complicated since different sets of animals were sampled at week 78 and thereafter than were studied

through week 52; also, a large range of values for serum enzymes of individual animals was observed.

The relative liver weight increase in high-dose females at each scheduled sacrifice was not accompanied by any significant histopathological correlates. Conversely, the histologic liver changes in males were for the most part minimal and were not accompanied by weight changes or consistent liver enzyme effects. In light of the fact that the test material has been shown to be a liver enzyme inducer, it does not appear the dose levels chosen were adequate for a carcinogenicity study.

A LEL for chronic toxicity can be set at 350 ppm in females (21.8 mg/kg/day) based on decreased mean body weights and weight gains and a decrease in the liver-to-body weight ratio and set at 350 ppm in males (15.6 mg/kg/day) based on an increase in fatty liver changes; the corresponding NOEL is 50 ppm (2.2 mg/kg/day in males and 2.7 mg/kg/day in females) cyproconazole fed in the diet for two years.

272 E

CASWELL FILE

007871

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

APR 23 1990

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: CYPROCONAZOLE - DOG CHRONIC FEEDING AND RAT CHRONIC FEEDING/ONCOGENICITY STUDIES

TO: SUSAN LEWIS/GRABLE
PRODUCT MANAGER (21)
REGISTRATION DIVISION (H7505C)

FROM: LINDA L. TAYLOR, PH.D. Linda L. Taylor 4/17/90
TOXICOLOGY BRANCH II, SECTION II
HEALTH EFFECTS DIVISION (H7509C)

THRU: K. CLARK SWENTZEL K. Clark Swentzel 4/18/90
SECTION II HEAD, TOXICOLOGY BRANCH II
HEALTH EFFECTS DIVISION (H7509C)

AND

MARCIA VAN GEMERT, PH.D. Marcia Gemert 4/19/90
CHIEF, TOXICOLOGY BRANCH/HFAS/HED (H7509C)

REGISTRANT: SANDOZ CROP PROTECTION CORPORATION
CHEMICAL: CYPROCONAZOLE
SYNONYMS: SAN 619 F
PROJECT: 9-2056
CASWELL NO.: 272E
MRID NO.: 412129-01 & 411647-01
RECORD NO.: NONE PROVIDED
IDENTIFYING NO.: 55947-RGG
ACTION REQUESTED: NONE. TWO CHRONIC STUDIES SUBMITTED.

COMMENT: IN A LETTER DATED JULY 10, 1989, THE REGISTRANT SUBMITTED THE TWO STUDIES REFERENCED ABOVE, IN ORDER TO PROVIDE THE AGENCY WITH A COMPLETE PICTURE OF THE CHRONIC TOXICOLOGY PACKAGE FOR SAN 619 F. A MOUSE ONCOGENICITY STUDY (MRID # 411472-01) WAS SUBMITTED UNDER SEPARATE COVER "FYI". THE DER FOR EACH (DOG AND RAT) STUDY IS ATTACHED.

DOG STUDY - THE ADMINISTRATION OF SAN 619 F TO BEAGLE DOGS FOR 52 WEEKS AT DOSE LEVELS OF 30, 100, AND 350 PPM [1.0, 3.2, AND 12.1 (MALES); 12.6 (FEMALES) MG/KG/DAY, RESPECTIVELY] RESULTED IN DIFFERENCES IN SEVERAL CLINICAL LABORATORY PARAMETERS BETWEEN THE CONTROL AND TREATED ANIMALS. ABSOLUTE AND RELATIVE LIVER WEIGHTS WERE INCREASED IN THE HIGH-DOSE ANIMALS OF BOTH SEXES COMPARED TO CONTROLS, BUT STATISTICAL SIGNIFICANCE WAS ATTAINED ONLY IN THE MALES. RELATIVE KIDNEY WEIGHT WAS INCREASED (SIGNIFICANTLY) IN BOTH THE LOW- AND HIGH-DOSE FEMALES. THE NOEL CAN BE SET AT 30 PPM (1.0 MG/KG/DAY) AND THE LEL AT 100 PPM (3.2 MG/KG/DAY), BASED ON LIVER EFFECTS.

CLASSIFICATION: CORE MINIMUM UNDER GUIDELINE 83-1, CHRONIC TOXICITY.

RAT STUDY - THE ADMINISTRATION OF SAN 619 F TO MALE RATS FOR 118 WEEKS AND TO FEMALE RATS FOR 121 WEEKS AT DOSE LEVELS OF 0, 20, 50, OR 350 PPM (MALES - 1.0, 2.2, AND 15.6 MG/KG; FEMALES - 1.2, 2.7, 21.8 MG/KG) RESULTED IN DECREASED BODY WEIGHTS IN HIGH-DOSE FEMALES AND INCREASED INCIDENCE OF FATTY INFILTRATION OF THE LIVER IN THE HIGH-DOSE MALES. THE NOEL FOR SYSTEMIC TOXICITY CAN BE SET AT 50 PPM, THE LEL AT 350 PPM, BASED ON DECREASED BODY WEIGHT IN FEMALES AND FATTY INFILTRATION IN THE LIVER OF MALES.

UNDER THE CONDITIONS OF THE STUDY, SAN 619 F WAS NOT CARCINOGENIC. BASED ON THE LACK OF: 1) ANY BIOLOGICALLY SIGNIFICANT BODY WEIGHT DECREMENT; 2) ANY SIGNIFICANT HISTOPATHOLOGICAL CORRELATE ACCOMPANYING THE INCREASED RELATIVE LIVER WEIGHT; 3) ANY INCREASE IN THE LIVER ENZYME ACTIVITIES IN THE FEMALES; 4) ANY CONSISTENT CHANGE IN THE LIVER ENZYME ACTIVITIES IN THE HIGH-DOSE MALES, SUGGESTS THAT THE DOSE LEVELS CHOSEN WERE NOT ADEQUATE TO DETERMINE THE CARCINOGENIC POTENTIAL OF THE TEST MATERIAL.

CLASSIFICATION: CORE SUPPLEMENTARY UNDER GUIDELINE 83-2, CARCINOGENICITY STUDY; CORE MINIMUM UNDER GUIDELINE 83-1, CHRONIC TOXICITY STUDY.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

DEC 1 1989

007632

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Cyproconazole-In Vitro Chromosome Assay with Chinese Hamster Ovary (CHO) Cells

TO: Lewis/Gable PM 21
Registration Division (H7509C)

FROM: K. Clark Swentzel, Section Head
Section II, Toxicology Branch II (HFAS)
HED (7509C)

K. Clark Swentzel 11/21/89

THRU: Marcia van Gemert, Ph.D.
Chief, Toxicology Branch II (HFAS)
HED (7509C)

Marcia van Gemert 11/22/89

EPA ID No. 55947-RGG
Acc. No. 411587-01
Project No. 9-1791
Caswell No. 272E
Registrant: Sandoz Corp.

Requested Action

Review subject study.

Response

The primary review of this study was performed by Dynamac Corp. and the secondary review was done by Dr. John Chen, Section I/Tox Branch II; the DER is attached. Cyproconazole was evaluated in a multiple-harvest cytogenetic assay for the potential to induce chromosome aberrations in Chinese hamster ovary cells. Concentrations of test material were 100, 150 or 200 ug/ml without S9 activation and 100, 150, 200 or 250 ug/ml with S9 activation; cultures were harvested at 4, 9 and 19 hours after treatment. A positive response was observed in both the S9 activated and nonactivated assays at different dose levels and at different harvest times. Therefore, it was concluded that Cyproconazole was clastogenic in this mammalian cell test system and that metabolic activation was not required to demonstrate the effect.

Classification: acceptable

1

(70)

EPA No.: 68D80056
DYNAMAC No.: 242-A
TASK No.: 2-42A
November 15, 1989

DATA EVALUATION RECORD

CYPROCONAZOLE

Mutagenicity--*In vitro* Chromosome Assay with Chinese Hamster Ovary (CHO) Cells

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 11/15/89

EPA No.: 68D80056
DYNAMAC No.: 242-A
TASK No.: 2-42A
November 15, 1989

DATA EVALUATION RECORD

CYPROCONAZOLE

Mutagenicity--In vitro Chromosome Assay with Chinese Hamster Ovary (CHO) Cells

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 11-15-1989

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 11-15-89

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J. Pienta
Date: 11/15/89

K. Clark Swentzel
EPA Reviewer and
Section Head, Section II
Toxicology Branch II
(H-7509C)

Signature: K. Clark Swentzel
Date: 11/20/89

DATA EVALUATION RECORD

CHEMICAL: Cyproconazole.

STUDY TYPE: In vitro chromosome assay with Chinese hamster ovary (CHO) cells.

MRID NUMBER: 411587-01.

TEST MATERIAL: Cyproconazole.

SYNONYM(S): 2-(4-Chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)-butan-2-ol; SAN 619F/94361-06-5, 94361-07-6.

SPONSOR: Sandoz Crop Protection Corporation, Basle, Switzerland.

TESTING FACILITY: Research and Consulting Company B.V., Hertogen basch, the Netherlands.

TITLE OF REPORT: Evaluation of the Ability of Cyproconazole to Induce Chromosome Aberrations in Cultured Chinese Hamster Ovary (CHO) Cells.

AUTHOR(S): Enninga, I.C.

STUDY NUMBER(S): 0883/ECC 153.

REPORT ISSUED: June 28, 1988.

CONCLUSIONS/EXECUTIVE SUMMARY:

Cyproconazole was evaluated in a multiple-harvest cytogenetic assay for the potential to induce chromosome aberrations in Chinese hamster ovary cells. Cultures exposed to 100, 150, or 200 µg/mL without S9 activation and cultures exposed to 100, 150, 200, or 250 µg/mL with S9 activation were harvested 4, 9, and 19 hours after treatment. Results indicated that significant increases in cells with aberrations were scored in both the nonactivated and S9-activated assays at different dose levels and at different harvest times. The effect was not dose or time dependent. Regardless of the condition, dose, or harvest interval, the frequency and type (chromatid breaks and acentric fragments) of aberrations that were induced were comparable. This finding, in conjunction with the narrow dose range, suggests that the clastogenic activity of cyproconazole occurs within a limited dose range. We conclude, from the positive response observed in the nonactivated and S9 activated assays, that cyproconazole was clastogenic in this mammalian cell test system and that metabolic activation was not required to demonstrate the effect.

Study Classification: The study is acceptable.

A. MATERIALS:

1. Test Material: Cyproconazole.
   Description: Light brown powder.
   Batch No.: 8507.
   Purity: 95.6 ± 1%.
   Contaminants: Not listed.
   Solubility: In water, 140 ± 4 ppm at 22°C; in ethanol and dimethylsulfoxide (DMSO) >23% at 25°C.
   Solvent Used: DMSO.
   Stability: Decomposition <5%/2 years at 20°C.
   Other Comments: The test material was stored at room temperature in the dark. Solutions of the test material were prepared immediately prior to use and were protected from light.

2. Cell Line: The Chinese hamster ovary (CHO) cells (subline CHO-K1) used in this assay were obtained from Dr. A. T. Natarajan, State University of Leiden, the Netherlands. Prior to use, the CHO cells were grown in Ham's F-10 medium supplemented with 10% fetal calf serum, L-glutamine, and antibiotics.

3. S9 Fraction: The S9 fraction was derived from the livers of young adult male Wistar or Sprague Dawley rats induced with Aroclor 1254. Prior to use, the concentration of cytochrome P-450 in the S9 fraction was determined and found to be 173.6 nM cytochrome P-450/g wet liver. The S9-cofactor mix contained 0.5 mL S9/mL of the reaction mixture.

4. Control Compounds: DMSO served as the negative control; mitomycin C (MMC) at 0.075 µg/mL was used as the nonactivated positive control and cyclophosphamide (CP) at 10 µg/mL was used as the S9-activated positive control.

B. STUDY DESIGN:

1. Preliminary Cytotoxicity Assay: Prepared cultures (four replicates), seeded at ≈5 x 10^5 cells/dish, were exposed for 2 hours to eight concentrations of the test material (1.0 to 2000 µg/mL) or the solvent control (DMSO) both in the presence and absence of S9 activation. Following exposure, cells were washed; cells from two of the four replicate cultures were counted to determine survival immediately following treatment. The remaining cultures were refed fresh medium and reincubated for 18 to 20 hours; cells were collected and counted, and the relative percent survival was determined.

2. Cytogenetic Assay:

a. Treatment: Based on the preliminary cytotoxicity assay results, doses were selected for the nonactivated and S9-activated cytogenetic assays. Duplicate cultures containing ≈4 x 10^6, ≈2 x 10^6, or ≈1 x 10^6 cells/flask were exposed for 2 hours to the selected doses of the test material, solvent (DMSO), or positive controls (2 x 10^6 cells/flask dosed with 0.075 µg/mL MMC/-S9 or 10 µg/mL CP/+S9).

Cultures were washed, refed with fresh medium, and reincubated for 4 hours (cultures containing ≈4 x 10^6 cells), 9 hours (cultures containing ≈2 x 10^6 cells), or 19 hours (cultures containing ≈1 x 10^6 cells). Colchicine (2 µg/mL) was added to cultures exposed to the highest test material dose or the positive controls 2 to 2.5 hours prior to cell harvest.

Metaphase cells were collected by mitotic shake-off, treated with hypotonic 1% sodium citrate, and fixed with methanol:acetic acid (3:1). Slides were stained with 5% Giemsa and coded.

b. Metaphase Analysis: Two hundred metaphase cells per treatment group (100/culture) were scored for structural chromosome aberrations. Numerical variations were noted but not included as aberrations.

3. Statistical Analysis: The data were evaluated for statistical significance at p values of 0.05, 0.01, and 0.001 by the Chi-square test.

4. Evaluation Criteria:

   a. Assay Validity: The assay was considered valid if 1) the number of chromosome aberrations in the solvent control cultures fell within the historical range of data recorded by the reporting laboratory and 2) the positive control chemicals induced a significant ($p<0.05$) increase in the number of cells with aberrations.

   b. Positive Response: The test material was considered positive if it induced a significant ($p<0.05$) and dose-related increase in the number of cells with chromosome aberrations. In the absence of a dose-response relationship, the test material was considered positive if it induced a significant effect that was reproducible.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: The eight nonactivated and S9-activated doses of the test material evaluated in the preliminary cytotoxicity assay ranged from 1 to 2000 µg/mL. Compound precipitation was observed in cultures containing the three highest doses (333, 1000, and 2000 µg/mL) with and without S9 activation. In the nonactivated test, no cells survived exposure to the three highest doses of the test material. At nonactivated doses ≤ 100 µg/mL, ≥ 84% of the cells survived. Under S9-activated conditions, the two highest doses were completely cytotoxic and less than 5% of the cells survived exposure to 333 µg/mL/+/-S9. Lower S9-activated doses (1 to 100 µg/mL) were relatively noncytotoxic. Based on these results, the cytotoxic assay was repeated using a narrower range of test material doses (100, 150, 200, and 250 µg/mL/+/-S9). In the absence of S9 activation, 8% of the cells exposed to 250 µg/mL were viable 18 to 20 hours posttreatment; at 200 µg/mL, 55% of the cells were recovered 18 to 20 hours posttreatment. The inclusion of S9 activation reduced cytoxicity as indicated by the 18- to 20-hour posttreatment recovery of 64% of the cells exposed to 250 µg/mL. The doses selected for the cytogenetic assay were 50, 100, 150, and 200 µg/mL/-S9 and 100, 150, 200, and 250 µg/mL/+S9.

2. Cytogenetic Assay:

a. Nonactivated Test: Under nonactivated conditions, statistically significant ($p<0.05$) increases in the number of cells with aberrations were observed in cultures harvested 4 and 9 hours postexposure to 200 µg/mL. A significant increase ($p<0.05$) in chromosome aberrations was also noted in the 100 µg/mL test group harvested 4 hours posttreatment. No significant effects were seen in cultures harvested at the 19-hour interval. Representative nonactivated results were selected from the 9-hour cell harvest and are presented in Table 1; reported results from the 4- and 19-hour fixation times are presented in Appendix A; study author's Tables Nos. 2 and 6, respectively.

b. S9-Activated Test: Metaphases were not available from the high-dose group (250 µg/mL) at the 4- and 9-hour harvest times. Significant increases in cells with aberrations were seen in the following test groups: 100 µg/mL (9- and 19-hour harvests), 150 µg/mL (4-hour harvest), 200 µg/mL (9-hour harvest), and 250 µg/mL (19-hour harvest). No dose- nor time-related trend was apparent. It was noted, however, that under both non-activated and S9-activated conditions, the predominant types of aberrations that were scored at doses inducing a significant effect were generally chromatid breaks and acentric fragments. S9-activated results from the 9-hour cell harvest were selected as representative and appear in Table 1; reported results from the 4- and 19-hour fixation times are presented in Appendix B; study author's Table Nos. 3 and 7, respectively.

Based on the combined results of the multiple-harvest nonactivated and S9-activated assays, the study author concluded that cyproconazole is "weakly clastogenic" in this test system.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study was properly conducted and that the study author interpreted the data correctly. Although cyproconazole did not induce a dose- or time-dependent clastogenic effect, significant increases were consistently seen under nonactivated and S9-activated conditions. Similarly, the type and frequency of aberrations were generally comparable regardless of the condition, dose, or harvest interval. It would, therefore, appear that the clastogenic activity of cyproconazole is confined to an extremely narrow range of reactive doses and that S9 activation was not required to demonstrate this effect.

TABLE 1. Representative Results of the Multiple-Harvest CHO Cell *in vitro* Cytogenetic Assay with Cyproconazole

Substance	Dose (µg/mL)	S9 activation	Harvest[a] Time (Hours)	No. of Cells Scored	No. Cells with Aberrations[b]	% Cells with Aberrations	Biologically Significant Aberrations[c] (No./Type)
Solvent control							
Dimethylsulfoxide	--	-	9	200	7	3.5	4TB; 1SB; 2AF
	--	+	9	200	5	2.5	1TB; 1SB; 4AF
Positive control							
Mitomycin C	0.075	-	9	200	18*	9.0	7TB; 2SB; 7AF; 3E
Cyclophosphamide	10	+	9	200	56**	28.0	22TB; 7SB; 24AF; 15E
Test Material							
Cyproconazole	100[d]	-	9	200	5	2.5	2TB; 4AF
	150	-	9	200	7	3.5	1SB; 5AF; 1D
	200	-	9	200	17*	8.5	10TB; 1SB; 7AF; 1E
	100[e]	+	9	200	12*	6.0	5TB; 2SB; 5AF; 1MA
	150	+	9	200	5	2.5	5TB
	200	+	9	200	12*	6.0	5TB; 1SB; 6AF; 2D

[a]Time after compound administration.

[b]Gaps not included.

[c]Abbreviations used:

TB - Chromatid break
SB - Chromosome break
D - Dicentric
AF - Acentric fragment
E - Exchange figure
MA - Multiple aberrations

[d]Significant increases in cells with aberrations were also noted in the 100- and 200-µg/mL nonactivated test groups harvested 4 hours postexposure.

[e]Significant increases in cells with aberrations were also noted in the following S9-activated test groups: 150 µg/mL at 4 hours and 100 and 250 µg/mL at 19 hours.

*Significantly different than the solvent control ($p<0.05$) by the X^2 test.

**Significantly different than the solvent control ($p<0.001$) by the X^2 test.

E. QUALITY ASSURANCE MEASURES: A quality assurance statement was signed and dated June 28, 1988.

F. CBI APPENDIX: Appendix A, Results from the 4- and 19-hour Nonactivated Fixative Times, CBI pp. 13 and 17; Appendix B, Results from the 4- and 19-hours S9-activated Fixative Times, CBI pp. 14 and 18; and Appendix C, Materials and Methods, CBI pp. 8-10.

APPENDIX A

Results from the 4- and 19-Hour Nonactivated Fixative Times

CBI pp. 13-17

CYPROCONAZOLE

Page_____ is not included in this copy.

Pages 81 through 82 are not included.

The material not included contains the following type of information:

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____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX B

Results from the 4- and 19-Hour S9-Activated Fixative Times

CBI pp. 14-18

CYPROCONAZOLE

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____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

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APPENDIX C

Methods and Materials

CBI pp. 8-10

CYPROCONAZOLE

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_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

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REVIEWED BY: LINDA L. TAYLOR, PH.D. Linda Lee Taylor 11/21/89
TOX. BRANCH II, SECTION II (H7509C)
SECONDARY REVIEWER: K. CLARK SWENTZEL K. Clark Swentzel 11/21/89
HEAD SECTION II, TOX. BRANCH II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: ONE-YEAR CHRONIC - DOG TOX. CHEM. NO.: 272E

MRID NO.: 412129-01

TEST MATERIAL: ALPHA-(4-CHLOROPHENYL)-ALPHA-(1-CYCLOPROPYLETHYL)-1H-1,2,4-TRIAZOLE-1-ETHANOL; SAN 619F

SYNONYMS: CYPROCONAZOLE

STUDY NUMBER: PROJECT NO. 394-D

SPONSOR: SANDOZ CROP PROTECTION CORPORATION

TESTING FACILITY: SANDOZ LTD. AGRO DEVELOPMENT - TOXICOLOGY DEPARTMENT BASLE/SWITZERLAND

TITLE OF REPORT: CHRONIC ORAL TOXICITY BY DIETARY ADMINISTRATION TO BEAGLE DOGS FOR ONE YEAR

AUTHOR(S): S.F.P. WARREN, F. HAMBURGER, S. CARPY, AND F. MILLER

REPORT ISSUED: APRIL 18, 1988

QUALITY ASSURANCE: A QUALITY ASSURANCE STATEMENT WAS PROVIDED.

CLASSIFICATION: CORE-MINIMUM.

CONCLUSION: THE ADMINISTRATION (DIET) OF SAN 619 F TO BEAGLE DOGS FOR 52 WEEKS AT DOSE LEVELS OF 30, 100, AND 350 PPM [1.0, 3.2, AND 12.1 (MALES); 12.6 (FEMALES) MG/KG/DAY, RESPECTIVELY] RESULTED IN DIFFERENCES IN SEVERAL CLINICAL LABORATORY PARAMETERS BETWEEN THE CONTROL AND TREATED ANIMALS, WHICH ARE CONSISTENT WITH EFFECTS ON THE LIVER (ELEVATED ALKALINE PHOSPHATASE AND ALAT LEVELS; DECREASED TOTAL PROTEIN, ALBUMIN, AND CHOLESTEROL LEVELS). ABSOLUTE AND RELATIVE LIVER WEIGHTS WERE INCREASED IN THE HIGH-DOSE ANIMALS OF BOTH SEXES COMPARED TO CONTROLS, BUT STATISTICAL SIGNIFICANCE WAS ATTAINED ONLY IN THE MALES. RELATIVE KIDNEY WEIGHT WAS INCREASED (SIGNIFICANTLY) IN BOTH THE LOW- AND HIGH-DOSE FEMALES.

STATISTICALLY SIGNIFICANT INCREASES WERE OBSERVED IN CYTOCHROME P_{450} IN BOTH SEXES OF THE HIGH-DOSE AND IN THE MID-DOSE FEMALES. LAMINAR EOSINOPHILIC INTRAHEPATOCYTIC BODIES WERE OBSERVED IN ALL HIGH-DOSE MALES, ONE MID-DOSE MALE, AND TWO HIGH-DOSE FEMALES AND WERE CONSIDERED TO REPRESENT ADAPTIVE HYPERTROPHY OF THE ENDOPLASMIC RETICULUM.

THE NOEL CAN BE SET AT 30 PPM (1.0 MG/KG/DAY) AND THE LEL AT 100 PPM (3.2 MG/KG/DAY), BASED ON LIVER EFFECTS.

A. MATERIALS:

1. TEST COMPOUND: SANDOZ 619F, TECHNICAL; DESCRIPTION: A LIGHT BROWN POWDER; BATCH #: 8507; PURITY: 95+ 1%; STABILITY: DOCUMENTED.

2. TEST ANIMALS: SPECIES: DOG; STRAIN: PUREBRED BEAGLES (CANIS FAMILIARIS); AGE: APPROXIMATELY 5 MONTHS; WEIGHT: MALES: 7.6-8.0 KG, FEMALES: 7.0-7.5 KG; SOURCE: MARSHALL FARMS BREEDING LABORATORIES, NORTH ROSE, NEW YORK.

B. STUDY DESIGN:

1. ANIMAL ASSIGNMENT

ANIMALS WERE ASSIGNED RANDOMLY TO THE FOLLOWING TEST GROUPS:

GROUP	DOSE IN DIET (PPM)	EXPOSURE PERIOD (52 MONTHS) MALES	FEMALES
1 CONTROL	0	4	4
2 LOW	30	4	4
3 MID	100	4	4
4 HIGH	350	4	4

2. DIET PREPARATION

THE TEST ARTICLE WAS ADMINISTERED IN THE DIET (POWDERED DIET # 24-335-1; KLINGENTALMUHLE, CH-BASLE). A DIET PREMIX WAS PREPARED MONTHLY [40 GRAMS OF SAN 619F MIXED WITH 3960 GRAMS OF POWDERED FODDER CONCENTRATION OF 10 MG SAN 619F PER GRAM (1%)] WHICH WAS THEN MIXED WEEKLY BY ADDING ADDITIONAL POWDERED FODDER TO MAKE THE APPROPRIATE DOSE LEVELS. THE CONTROLS RECEIVED UNTREATED DIET. EACH DOG WAS OFFERED APPROXIMATELY 400 GRAMS OF FOOD DAILY AND WATER AD LIBITUM.

RESULTS

DATA PROVIDED INDICATE THE DIETS TO BE WITHIN +/-10% OF THE TARGET CONCENTRATIONS THROUGHOUT THE STUDY.

3. STATISTICS - THE PROCEDURES UTILIZED IN ANALYZING THE NUMERICAL DATA ARE EXPLAINED ON PAGE 17 OF THE FINAL REPORT (COPY ATTACHED).

C. METHODS AND RESULTS:

1. OBSERVATIONS

ALL DOGS WERE OBSERVED TWICE DAILY FOR SIGNS OF ILL HEALTH, AND THE NOSE, EYES, ANUS, AND BUCCAL CAVITY WERE CHECKED DAILY.

RESULTS:

TOXICITY/MORTALITY (SURVIVAL)

THERE WERE NO DEATHS OR UNSCHEDULED SACRIFICES DURING THE STUDY.

CLINICAL OBSERVATIONS

SUBDUED BEHAVIOR (DURING WEEKS 4 TO 8) AND LOWER BODY-WEIGHT GAIN (DURING THE FIRST 4 WEEKS) WERE OBSERVED IN ONE HIGH-DOSE FEMALE. FOOD CONSUMPTION ALSO TENDED TO BE LOW IN THIS ANIMAL UP TO WEEK 10. THEREAFTER, THIS ANIMALS DISPLAYED COMPARABLE BEHAVIOR TO THE OTHER DOGS. ONE MID-DOSE FEMALE DISPLAYED SUBDUED BEHAVIOR WITH BODY WEIGHT LOSS AND LOW FOOD CONSUMPTION DURING WEEK 2.

ONE HIGH-DOSE FEMALE FAILED TO COME INTO HEAT DURING THE STUDY, AND TERMINAL HISTOLOGICAL EXAMINATION REVEALED IMMATURE OVARIES.

2. BODY WEIGHT AND FOOD CONSUMPTION

ANIMALS WERE WEIGHED WEEKLY AND FOOD CONSUMPTION WAS DETERMINED WEEKLY. GROUP MEAN WEEKLY INTAKE OF TEST MATERIAL WAS CALCULATED FROM INDIVIDUAL BODY WEIGHT AND FOOD CONSUMPTION.

RESULTS

THE AUTHOR REPORTED AN INITIAL EFFECT ON BODY-WEIGHT GAIN FOR THE HIGH-DOSE MALES; A DECREASE WAS NOTED INITIALLY, WHICH WAS GREATEST AT WEEK 9 AND THEN BECAME COMPARABLE TO CONTROL THEREAFTER.

BODY WEIGHT (% CONTROL)

WEEK MALES	0	1	3	6	9	14	15	16	26	50
LOW	96	100	100	97	97	97	98	98	102	117
MID	101	104	105	103	103	103	104	105	104	114
HIGH	100	100	99	95	94	93	93	93	97	105
WEEK FEMALES	0	1	3	6	9	14	27	44	47	50
LOW	103	100	101	98	100	100	104	98	95	101
MID	101	101	100	100	107	111	107	107	104	111
HIGH	96	93	94	90	95	98	99	93	92	93

NOTE: DUE TO A COMPUTER PROBLEM AT THE TESTING FACILITY, PRE-TREATMENT VALUES FOR BOTH BODY WEIGHT AND FOOD CONSUMPTION WERE NOT AVAILABLE.

BODY-WEIGHT GAIN (KG)

	WEEK 0 - 9	WEEK 0 - 13	WEEK 13 - 26	WEEK 26 - 52	WEEK 0 - 52
MALES					
C	1.9	2.3	1.1	-0.1	3.3
L	1.9	2.5	1.4	1.5	5.4
M	2.1	2.7	1.1	0.7	4.5
H	1.3	1.8	1.3	0.5	3.6
FEMALES					
C	1.0 (1.0)*	1.1	0.6	0.5	2.2
L	0.8 (0.6)	0.9	0.6	0.5	2.0
M	1.5 (0.9)	1.9	0.4	0.9	3.2
H	0.9 (0.5)	1.1	0.7	0.0	1.8

* VALUE FOR BODY-WEIGHT GAIN BETWEEN WEEKS 0 AND 6

IN GENERAL, MEAN FOOD CONSUMPTION OF ALL OF THE MALE GROUPS WAS SIGNIFICANTLY GREATER THAN CONTROL AT VARIOUS TIME POINTS DURING THE STUDY. FOR FEMALES, FOOD CONSUMPTION VARIED CONSIDERABLY, WITH THE HIGH-DOSE GROUP SHOWING DECREASED INTAKE, COMPARED TO THE CONTROLS AND OTHER TREATED GROUPS FOR THE FIRST 3 WEEKS OF THE STUDY AND DURING WEEKS 31, 36, 42, 47, AND 51 (NOT STATISTICALLY SIGNIFICANT). THE LOW-DOSE FEMALES SHOWED DECREASED FOOD CONSUMPTION FROM WEEK 23 TO WEEK 48 (STATISTICALLY SIGNIFICANT AT WEEK 26 ONLY), AND THE MID DOSE SHOWED A DECREASE AT WEEKS 31, 36, 37, AND DURING WEEKS 42-47 (NONE STATISTICALLY SIGNIFICANT BUT $\geq$ 10% BELOW CONTROL VALUE).

FOOD EFFICIENCY DATA PROVIDED IN THE REPORT INDICATE THAT THERE WAS A SLIGHTLY REDUCED EFFICIENCY AT THE HIGH DOSE FOR MALES OVER THE FIRST 26 WEEKS. FOR THE FIRST 9 WEEKS, BOTH SEXES AT THE HIGH DOSE DISPLAYED A REDUCED FOOD EFFICIENCY (NO STATISTICS WERE PERFORMED ON THESE DATA).

3. COMPOUND INTAKE

AVERAGE DAILY DOSES OF THE TEST MATERIAL WERE CALCULATED FROM NOMINAL DIETARY CONCENTRATIONS, INDIVIDUAL BODY WEIGHT, AND FOOD CONSUMPTION.

	CONSUMED (MG/KG/DAY)	
	MALES	FEMALES
LOW	0.99	0.99
MID	3.15	3.23
HIGH	12.05	12.58

4. OPHTHALMOLOGICAL EXAMINATIONS

OCULAR EXAMINATIONS WERE PERFORMED ON ALL OF THE DOGS PRIOR TO TREATMENT AND DURING WEEK 50, USING A FUNDUS CAMERA FOLLOWING ADMINISTRATION OF A MYDRIATIC.

RESULTS

THERE WERE NO TREATMENT-RELATED OCULAR CHANGES IN EITHER SEX.

5. BLOOD ANALYSIS

CLINICAL LABORATORY STUDIES WERE CONDUCTED ON ALL DOGS (FASTED 16-18 HOURS) PRIOR TO STUDY INITIATION (WEEKS -1 & -2), AT 3, 6, AND 9 MONTHS, AND AT STUDY TERMINATION. THE CHECKED (X) PARAMETERS WERE EXAMINED.

A. HEMATOLOGY

X	HEMATOCRIT (HCT)*	X	LEUKOCYTE DIFFERENTIAL COUNT*
X	HEMOGLOBIN (HGB)*	X	MEAN CORPUSCULAR HGB (MCH)
X	LEUKOCYTE COUNT (WBC)*	X	MEAN CORPUSCULAR HGB CONC. (MCHC)
X	ERYTHROCYTE COUNT (RBC)*	X	MEAN CORPUSCULAR VOLUME (MCV)
X	PLATELET COUNT*	X	RETICULOCYTE COUNT
	THROMBOCYTE COUNT		METHEMOGLOBIN
X	PROTHROMBIN TIME		HEINZ BODIES
	ERYTHROCYTE INDICES		HOWELL-JOLLY BODIES

* REQUIRED FOR CHRONIC STUDIES

RESULTS

INCREASED PLATELET COUNTS WERE OBSERVED AT THE HIGH DOSE IN BOTH SEXES THROUGHOUT THE STUDY. MID-DOSE MALES ALSO DISPLAYED MARGINALLY INCREASED COUNTS AT WEEKS 26, 38, AND 52. PROTHROMBIN TIME WAS MEASURED AT WEEK 48 TO ASSESS WHETHER THE DISTURBED PLATELET COUNT WAS ASSOCIATED WITH DISTURBED CLOTTING FUNCTION. THE INCREASE IN PROTHROMBIN TIME OBSERVED WAS CONSIDERED (BY THE AUTHOR) TO BE THE RESULT OF THE OCCASIONAL LOW VALUES IN THE LOW-DOSE AND CONTROL GROUPS, AND NOT TO TREATMENT. HOWEVER, IT IS TO BE NOTED THAT THE LOW- AND MID-DOSE FEMALES SHOWED LOWER VALUES THAN THE CONTROL FEMALES.

HEMATOLOGICAL VALUES (% OF CONTROL)

PARAMETER	GROUP	WEEK -1	WEEK 13	WEEK 26	WEEK 38	WEEK 48	WEEK 52
MALES							
PLATELETS TH/CMM	CONTROL	403	305	287	269	291	274
	LOW	334(83)	269(88)	256(89)	248(92)	266(91)	258(94)
	MID	386(96)	335(110)	356(124)	307(114)	332(114)	318(116)
	HIGH	358(89)	461(151)	423(147)	384(143)	409(140)	383(140)
PROTHROMB. SEC.	CONTROL					9.60	
	LOW					10.50(109)	
	MID					10.80(113)	
	HIGH					10.85(113)	
FEMALES	GROUP	WEEK 0	WEEK 13	WEEK 26	WEEK 38	WEEK 48	WEEK 52
PLATELETS TH/CMM	CONTROL	374	319	324	323	310	305
	LOW	352(94)	291(91)	317(98)	308(95)	322(104)	324(106)
	MID	362(97)	344(108)	351(108)	328(102)	371(120)	332(109)
	HIGH	344(92)	456(143)*	433(134)	415(129)	442(143)*	438(144)**
PROTHROMB. SEC.	CONTROL					10.08	
	LOW					9.88(98)	
	MID					9.83(98)	
	HIGH					11.05(110)	

B. CLINICAL CHEMISTRY

THE CHECKED (X) PARAMETERS WERE MEASURED.

	ELECTROLYTES:
X	CALCIUM*
X	CHLORIDE*
	MAGNESIUM*
X	PHOSPHORUS*
X	POTASSIUM*
X	SODIUM*

	ENZYMES
X	ALKALINE PHOSPHATASE
	CHOLINESTERASE#
X	CREATININE PHOSPHOKINASE*
X	LACTIC ACID DEHYDROGENASE
X	SERUM ALANINE AMINOTRANSFERASE (ALSO SGPT)*
X	SERUM ASPARTATE AMINOTRANSFERASE (ALSO SGOT)*
	GAMMA GLUTAMYL TRANSPEPTIDASE
X	GLUTAMATE DEHYDROGENASE

	OTHER:
X	ALBUMIN*
X	BLOOD CREATININE*
X	BLOOD UREA NITROGEN*
X	CHOLESTEROL*
X	GLOBULINS
X	GLUCOSE*
X	TOTAL BILIRUBIN*
X	TOTAL SERUM PROTEIN*
X	TRIGLYCERIDES
	SERUM PROTEIN ELECTROPHORESIS
X	GAMMA GLUTAMYLTRANSFERASE
X	LEUCINE ARYLAMIDASE

* REQUIRED FOR CHRONIC STUDIES
\# SHOULD BE REQUIRED FOR OP

RESULTS

THERE WAS A STATISTICALLY SIGNIFICANT DECREASE IN TOTAL PROTEIN, ALBUMIN, AND CHOLESTEROL VALUES AT VARIOUS TIME POINTS THROUGHOUT THE STUDY IN BOTH SEXES AT THE HIGH-DOSE. TOTAL BILIRUBIN VALUES ALSO TENDED TO BE DEPRESSED, ESPECIALLY IN THE HIGH-DOSE FEMALES, BUT STATISTICAL SIGNIFICANCE WAS NOT ATTAINED. ALKALINE PHOSPHATASE VALUES WERE INCREASED IN BOTH SEXES OF THE HIGH DOSE THROUGHOUT THE STUDY (STATISTICALLY SIGNIFICANT AT ALL TIME POINTS IN THE FEMALE, BUT ONLY AT WEEK 51 FOR MALES). THE MID-DOSE FEMALES DISPLAYED A SIMILAR INCREASE OF LESSER MAGNITUDE, BUT STATISTICAL SIGNIFICANCE WAS NOT ATTAINED. THE MID-DOSE MALES DISPLAYED AN INCREASE ONLY AT THE 51-WEEK TIME POINT, WHICH WAS NOT STATISTICALLY SIGNIFICANT. CALCIUM LEVELS WERE DECREASED ($p<0.01$) AT THE 13-WEEK TIME POINT IN BOTH SEXES OF THE HIGH DOSE AND AT THE 25-WEEK TIME POINT IN THE HIGH-DOSE MALES. ALAT VALUES FOR THE HIGH-DOSE MALES WERE ELEVATED AT ALL TIME POINTS DURING THE STUDY COMPARED WITH CONTROL VALUES, BUT STATISTICAL SIGNIFICANCE WAS ATTAINED ONLY AT WEEK 51. SIMILAR EFFECTS WERE NOT OBSERVED IN THE FEMALES. GLOB % WAS ELEVATED SIGNIFICANTLY IN THE HIGH-DOSE (BOTH SEXES) AT WEEKS 13 AND 25, AND IN THE HIGH-DOSE MALES AT WEEK 38. TRIGLYCERIDE VALUES WERE SIGNIFICANTLY DEPRESSED IN THE HIGH-DOSE FEMALE AT WEEKS 13 AND 38.

CLINICAL CHEMISTRY VALUES (% OF CONTROL)

PARAMETER	GROUP	WEEK -1	WEEK 13	WEEK 25	WEEK 38	WEEK 51
MALES						
T. PROTEIN G/L	CONTROL	59	65	69	70	67
	LOW	58(98)	63(97)	67(98)	68(97)	66(99)
	MID	58(98)	62(96)	69(101)	67(95)	66(98)
	HIGH	58(99)	56(86)**	61(89)**	59(85)**	62(92)
ALBUMIN G/L	CONTROL	25.7	28.6	31.3	30.1	32.9
	LOW	25.7(100)	27.8(97)	30.1(96)	28.4(94)	31.0(94)
	MID	25.3(98)	26.9(94)	29.0(93)	27.0(90)	30.3(92)
	HIGH	23.6(92)	21.2(76)**	23.6(76)**	20.2(67)**	25.6(78)**
CHOLESTEROL MMOL/L	CONTROL	2.89	3.82	3.29	3.48	3.59
	LOW	2.66(92)	2.96(78)	2.93(89)	3.04(87)	3.38(94)
	MID	2.39(83)	2.76(72)	2.80(85)	2.56(74)	3.05(85)
	HIGH	2.37(82)	1.38(36)**	1.80(55)	1.72(49)*	2.07(58)
BILIRUBIN UMOL/L	CONTROL	1.453	1.557	1.570	1.608	1.648
	LOW	1.495(103)	1.403(90)	1.648(105)	2.140(133)	1.708(104)
	MID	1.413(97)	1.420(91)	0.918(58)	1.713(107)	1.570(95)
	HIGH	1.565(108)	1.185(76)	1.500(96)	1.300(81)	1.340(81)
ALKALINE PHOSPHATASE U/L	CONTROL	403.3	298.0	137.8	126.6	96.1
	LOW	273.0(65)	169.0(57)	105.4(76)	107.4(85)	80.5(84)
	MID	299.5(69)	206.3(69)	140.8(102)	128.8(102)	117.4(122)
	HIGH	268.0(61)	380.3(128)	287.8(209)	362.3(286)	375.5(391)**
ALAT U/L	CONTROL	20.28	21.77	27.98	32.53	28.85
	LOW	24.38(120)	25.15(116)	28.10(100)	29.48(91)	27.00(94)
	MID	21.03(104)	25.08(115)	27.43(98)	27.83(86)	27.13(94)
	HIGH	19.10(94)	38.58(177)	40.05(143)	46.85(144)	48.57(16)
CALCIUM MMOL/L	CONTROL	2.740	2.698	2.675	2.545	2.593
	LOW	2.725(99)	2.608(97)	2.543(95)	2.425(95)	2.505(97)
	MID	2.628(96)	2.578(96)	2.528(95)	2.403(94)	2.483(96)
	HIGH	2.673(98)	2.408(89)**	2.400(90)**	2.353(92)	2.473(95)
GLOB. % %	CONTROL	56.55	56.11	54.68	57.14	51.17
	LOW	55.78(99)	55.82(99)	55.22(101)	58.22(102)	53.38(104)
	MID	56.26(99)	56.94(101)	58.23(106)	59.31(104)	54.06(106)
	HIGH	59.64(105)	62.14(111)*	61.50(112)*	65.99(115)*	58.56(114)

PARAMETER	GROUP	WEEK -1	WEEK 13	WEEK 25	WEEK 38	WEEK 51
FEMALES						
T. PROTEIN G/L	CONTROL	58.4	62.3	63.9	72	65.2
	LOW	59.8(102)	62.3(100)	68.1(107)	65.5(91)	64.7(99)
	MID	55.6(95)	59.4(95)	62.2(97)	63.6(88)	62.9(97)
	HIGH	59.9(103)	54.4(87)*	59.1(92)	58.3(81)	59.3(91)
ALBUMIN G/L	CONTROL	27.7	29.4	30.2	30.1	32.0
	LOW	27.3(99)	30.4(103)	29.6(98)	29.8(99)	30.7(96)
	MID	24.6(89)	28.3(96)	28.5(94)	27.9(93)	29.7(93)
	HIGH	26.9(97)	21.5(73)**	24.3(80)**	23.6(78)*	26.4(83)**
CHOLESTEROL MMOL/L	CONTROL	3.03	4.23	4.11	4.75	5.35
	LOW	2.87(94)	3.49(82)	5.19(126)	3.53(74)	4.56(85)
	MID	2.44(80)	3.98(94)	3.79(92)	4.79(101)	4.59(86)
	HIGH	2.51(83)	2.05(48)**	2.76(67)	2.45(52)**	2.80(52)*

PARAMETER	GROUP	WEEK -1	WEEK 13	WEEK 25	WEEK 38	WEEK 51
BILIRUBIN UMOL/L	CONTROL	1.815	2.013	1.925	2.550	2.090
	LOW	1.743(96)	1.950(97)	1.620(84)	1.955(77)	1.860(89)
	MID	1.673(92)	1.703(85)	1.320(69)	2.265(89)	1.803(86)
	HIGH	1.933(107)	1.368(68)	1.528(79)	1.455(57)	1.393(67)
ALKALINE PHOSPHATASE U/L	CONTROL	226.0	146.6	122.0	137.5	108.9
	LOW	197.8(88)	139.8(95)	105.6(87)	118.7(86)	98.6(91)
	MID	229.0(101)	216.8(148)	164.5(135)	158.5(115)	152.8(140)
	HIGH	248.3(110)	407.8(278)*	366.8(301)**	407.8(297)*	342.8(316)**
ALAT U/L	CONTROL	22.48	17.75	19.58	21.65	20.88
	LOW	21.08(94)	20.53(116)	16.30(83)	22.75(105)	17.80(85)
	MID	23.65(105)	20.90(118)	21.55(110)	22.65(105)	22.20(106)
	HIGH	22.23(99)	21.45(121)	22.13(113)	22.43(109)	22.85(109)
CALCIUM MMOL/L	CONTROL	2.628	2.665	2.465	2.323	2.540
	LOW	2.703(103)	2.635(99)	2.530(103)	2.348(94)	2.548(100)
	MID	2.603(99)	2.630(99)	2.455(100)	2.308(94)	2.578(101)
	HIGH	2.620(100)	2.455(92)**	2.318(94)	2.193(89)	2.365(93)
GLOB. % %	CONTROL	52.65	52.78	52.74	57.25	50.72
	LOW	54.44(103)	51.20(97)	56.52(107)	54.41(95)	52.56(104)
	MID	55.79(106)	52.39(99)	54.21(103)	56.12(98)	52.63(104)
	HIGH	55.18(105)	60.54(115)**	58.91(112)*	59.35(104)	55.43(109)

* $P<0.05$
** $P<0.01$

6. URINALYSIS

URINE WAS COLLECTED (AFTER A 16-18 HOUR FAST) FROM ALL ANIMALS PRE-DOSE, AND AT 3, 6, AND 9 MONTHS, AND PRIOR TO STUDY TERMINATION. THE CHECKED (X) PARAMETERS WERE EXAMINED.

	APPEARANCE*	X	GLUCOSE*
	VOLUME*	X	KETONES*
X	SPECIFIC GRAVITY*	X	BILIRUBIN*
X	pH	X	BLOOD*
X	SEDIMENT (MICROSCOPIC)*		NITRATE
X	PROTEIN*	X	UROBILINOGEN
	OSMOLALITY		

* REQUIRED FOR CHRONIC STUDIES

RESULTS

THERE WERE SPORADIC STATISTICALLY SIGNIFICANT ALTERATIONS IN A FEW URINARY PARAMETERS COMPARED TO CONTROL VALUES, BUT NONE THAT COULD BE ATTRIBUTED TO TREATMENT. THE pH OF THE HIGH-DOSE MALES WAS MORE ALKALINE THAN THE CONTROL VALUE AT WEEKS 12, 25, AND 38, BUT STATISTICAL SIGNIFICANCE WAS NOT ATTAINED.

GROSS PATHOLOGY

ALL ANIMALS WERE SACRIFICED AT STUDY TERMINATION AND WERE SUBJECTED TO GROSS PATHOLOGICAL EXAMINATION. ALL DOGS WERE EXAMINED EXTERNALLY BOTH VISUALLY AND

BY PALPATION, WITH PARTICULAR ATTENTION BEING PAID TO THE EYES, NOSE, BUCCAL CAVITY, EXTERNAL GENITALIA, AND ANUS. A MACROSCOPIC EXAMINATION WAS PERFORMED AFTER OPENING ALL CAVITIES AND OBSERVING THE TISSUES IN SITU. THE FOLLOWING ORGANS WERE WEIGHED:

ADRENAL	LIVER	SPLEEN
BRAIN	OVARIES	TESTES
KIDNEY	PITUITARY	THYROID WITH PARATHYROID

RESULTS

TWO HIGH-DOSE MALES DISPLAYED ENLARGED LIVERS AT NECROPSY, WITH PRONOUNCED LOBULAR PATTERNING. NO OTHER TREATMENT-RELATED FINDINGS WERE REPORTED.

ABSOLUTE AND RELATIVE LIVER WEIGHT WERE SIGNIFICANTLY INCREASED IN THE HIGH-DOSE MALES COMPARED TO CONTROLS. HIGH-DOSE FEMALES ALSO DISPLAYED AN INCREASE IN LIVER WEIGHT (BOTH ABSOLUTE AND RELATIVE), BUT A $P<0.05$ WAS NOT ATTAINED.

ABSOLUTE KIDNEY WEIGHT WAS SLIGHTLY INCREASED IN THE LOW- AND HIGH-DOSE FEMALES, BUT STATISTICAL SIGNIFICANCE WAS NOT ATTAINED. RELATIVE KIDNEY WEIGHT WAS SIGNIFICANTLY INCREASED IN BOTH THE LOW- AND HIGH-DOSE FEMALE GROUPS.

ABSOLUTE THYROID WEIGHT OF THE FEMALES SHOWED A SLIGHT INCREASE WITH DOSE, BUT THIS WAS NOT DOSE-RELATED. RELATIVE THYROID WEIGHT INCREASED WITH INCREASING DOSE, BUT THIS DID NOT ATTAIN STATISTICAL SIGNIFICANCE.

THE PITUITARY WAS ALSO FOUND TO BE HEAVIER IN THE HIGH-DOSE ANIMALS COMPARED TO THE RESPECTIVE CONTROLS, BUT STATISTICAL SIGNIFICANCE WAS NOT ATTAINED.

ORGAN WEIGHTS

	LIVER		KIDNEY		THYROID		PITUITARY	
	ABSOLUTE GRAMS	RELATIVE %	ABSOLUTE GRAMS	RELATIVE %	ABSOLUTE GRAMS	RELATIVE %	ABSOLUTE GRAMS	RELATIVE %
MALES								
CONTROL	251	2.34	45.0	0.43	0.79	0.0073	61.8	0.57
LOW	279	2.26	54.3	0.44	0.75	0.0061	84.8	0.69
MID	296	2.47	50.5	0.42	0.73	0.0061	67.0	0.56
HIGH	400**	3.64*	53.8	0.49	0.86	0.0077	75.3	0.68
FEMALES								
CONTROL	258	2.76	33.8	0.36	0.66	0.0070	89.5	0.96
LOW	297	3.24	37.4	0.41*	0.67	0.0073	82.5	0.89
MID	282	2.78	34.7	0.36	0.82	0.0080	70.8	0.69
HIGH	290	3.39	36.7	0.43*	0.77	0.0091	111.0	1.31

* $P<0.05$
** $P<0.01$

LIVER CHEMISTRY

AT NECROPSY, LIVER SAMPLES WERE TAKEN FROM ALL DOGS FOR ANALYSIS OF P_{450} CONTENT, GLUTATHIONE CONTENT (GSH), GLUTATHIONE-S-TRANSFERASE ACTIVITY (GST), AND P-AMINOPHENOL-HYDROXYLASE ACTIVITY (PAP).

RESULTS

HIGH-DOSE DOGS OF BOTH SEXES AND MID-DOSE FEMALES DISPLAYED STATISTICALLY SIGNIFICANT INCREASES IN CYTOCHROME P_{450} (SEE ATTACHED SPECIAL LIVER CHEMISTRY TABLE). MEASUREMENT OF PAP INDICATED NO INDUCTION OF ACTIVITY WITH INCREASING DOSE (ALTHOUGH THE LOW-DOSE FEMALE VALUE WAS SIGNIFICANTLY ELEVATED ABOVE CONTROL VALUE BY THE DUNNETT'S T-TEST PERFORMED BY THIS REVIEWER). THE AUTHOR INDICATED THAT SINCE PAP SHOWS A SLIGHTLY GREATER SPECIFICITY OF INDUCTION POTENTIAL WITH P_{448} INDUCERS, THE DATA SUGGEST THAT THE TEST MATERIAL IS A P_{450} INDUCER AND NOT A P_{448} INDUCER (OFTEN A MARKER OF CARCINOGENIC POTENTIAL). THERE WAS NO DOSE-RELATED EFFECT REPORTED FOR EITHER SEX ON GSH OR GST, ALTHOUGH STATISTICAL SIGNIFICANCE WAS REPORTED IN THE MID-DOSE ANIMALS OF BOTH SEXES FOR GSH. THIS REVIEWER FOUND A DOSE-RELATED DECREASE ($P<0.05$) IN GST VALUES IN THE MID- AND HIGH-DOSE FEMALES USING DUNNETT'S T-TEST.

HISTOPATHOLOGY

THE FOLLOWING CHECKED (X) TISSUES/ORGANS WERE COLLECTED FROM ALL ANIMALS.

	DIGESTIVE SYSTEM
	TONGUE
X	SALIVARY GLANDS*
X	ESOPHAGUS*
X	STOMACH*
X	DUODENUM*
X	JEJUNUM*
X	ILEUM*
X	CECUM*
X	COLON*
X	RECTUM*
X	LIVER*†
X	GALL BLADDER*
X	PANCREAS*
	RESPIRATORY
X	TRACHEA*
X	LUNG*
	NOSE
	PHARYNX
	LARYNX

	CARDIOVASC./HEMAT.
X	AORTA*
X	HEART*
	BONE MARROW*
X	LYMPH NODES*
X	SPLEEN*
X	THYMUS*
	UROGENITAL
X	KIDNEYS*†
X	URINARY BLADDER*
X	TESTES*†
X	EPIDIDYMIDES
X	PROSTATE
X	SEMINAL VESICLE
X	OVARIES*†
X	UTERUS*
	CERVIX
X	VAGINA

	NEUROLOGIC
X	BRAIN*†
X	PERIPH. NERVE* (SCIATIC)
X	SPINAL CORD (3 LEVELS)*
X	PITUITARY*
X	EYES (OPTIC N.)*
	GLANDULAR
X	ADRENALS*
	LACRIMAL GLAND
X	MAMMARY GLAND*
X	PARATHYROIDS*††
X	THYROIDS*††
	OTHER
X	BONE* (STERNUM/FEMUR)
X	SKELETAL MUSCLE*
X	SKIN*
X	ALL GROSS LESIONS AND MASSES*
	HEAD

* REQUIRED FOR CHRONIC STUDIES
† ORGAN WEIGHTS REQUIRED IN CHRONIC STUDIES/†† FOR NON-RODENT STUDIES

RESULTS

THE LIVER WAS SHOWN TO BE THE TARGET ORGAN. LAMINAR EOSINOPHILIC INTRAHEPATOCYTIC BODIES WERE OBSERVED IN ALL HIGH-DOSE MALES AND IN ONE MID-DOSE MALE. TWO HIGH-DOSE FEMALES ALSO DISPLAYED THESE BODIES. TWO HIGH-DOSE MALES DISPLAYED CANALICULAR BILE PLUGS, AND ONLY TREATED MALES SHOWED AN INCREASE IN INTRAHEPATIC PIGMENT.

OTHER OBSERVATIONS INCLUDE ONE HIGH-DOSE FEMALE WITH IMMATURE OVARIES AND ONE MID-DOSE MALE WITH DEGENERATION OF THE TESTICULAR GERMINAL EPITHELIUM. SINCE THIS FEMALE HAD NOT SHOWN ANY SIGN OF ESTROUS CYCLING DURING THE STUDY, THE AUTHOR STATED THAT IT WOULD BE IMPRUDENT TO DISMISS THE POSSIBILITY OF THIS FINDING BEING A CONSEQUENCE OF STEROID BIOSYNTHESIS INHIBITION.

CONCLUSION:

THERE WERE NO DIFFERENCES OBSERVED IN SURVIVAL, OPHTHALMOSCOPIC PARAMETERS, OR HEMATOLOGIC PARAMETERS FOLLOWING THE ADMINISTRATION (DIET) OF SAN 619 F TO BEAGLE DOGS FOR 52 WEEKS AT DOSE LEVELS OF 30, 100, AND 350 PPM [1.0, 3.2, AND 12.1 (MALES); 12.6 (FEMALES) MG/KG/DAY, RESPECTIVELY].

BODY WEIGHT GAIN WAS LOWER IN THE HIGH-DOSE ANIMALS COMPARED WITH CONTROL, ALTHOUGH THE MAGNITUDE OF THE DIFFERENCE WAS NOT GREAT. THERE WERE DOSE-RELATED INCREASES IN PLATELETS THROUGHOUT THE STUDY IN BOTH SEXES, ALTHOUGH STATISTICAL SIGNIFICANCE WAS NOT ALWAYS ATTAINED. PROTHROMBIN TIMES WERE CHECKED AT WEEK 48 AND WERE SLIGHTLY ELEVATED IN THE MID-DOSE MALES AND THE HIGH-DOSE ANIMALS.

DIFFERENCES OBSERVED IN SEVERAL CLINICAL LABORATORY PARAMETERS BETWEEN THE CONTROL AND TREATED ANIMALS ARE CONSISTENT WITH EFFECTS ON THE LIVER (ELEVATED ALKALINE PHOSPHATASE AND ALAT LEVELS; DECREASED TOTAL PROTEIN, ALBUMIN, AND CHOLESTEROL LEVELS).

ABSOLUTE AND RELATIVE LIVER WEIGHTS WERE INCREASED IN THE HIGH-DOSE ANIMALS OF BOTH SEXES COMPARED TO CONTROLS, BUT STATISTICAL SIGNIFICANCE WAS ATTAINED ONLY IN THE MALES. RELATIVE KIDNEY WEIGHT WAS INCREASED (SIGNIFICANTLY) IN BOTH THE LOW- AND HIGH-DOSE FEMALES.

STATISTICALLY SIGNIFICANT INCREASES WERE OBSERVED IN CYTOCHROME P_{450} IN BOTH SEXES OF THE HIGH-DOSE AND IN THE MID-DOSE FEMALES. LAMINAR EOSINOPHILIC INTRAHEPATOCYTIC BODIES WERE OBSERVED IN ALL HIGH-DOSE MALES, ONE MID-DOSE MALE, AND TWO HIGH-DOSE FEMALES AND WERE CONSIDERED TO REPRESENT ADAPTIVE HYPERTROPHY OF THE ENDOPLASMIC RETICULUM.

THE NOEL CAN BE SET AT 30 PPM (1.0 MG/KG/DAY) AND THE LEL AT 100 PPM (3.2 MG/KG/DAY), BASED ON LIVER EFFECTS.

CYPROCONAZOLE

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Pages 101 through 103 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

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____ Description of quality control procedures.

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____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

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Reviewed by: K. Clark Swentzel K. Clark Swentzel 9/15/88
Section 2 , Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. Marcia van Gemert 9/30/88
Section 2 , Tox. Branch (TS-769C)

007003

DATA EVALUATION REPORT

STUDY TYPE: 13-Week Feeding Study TOX. CHEM. NO.: 272E

MRID NO.: 406243-04

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol

SYNONYMS: Cyproconazole; SAN 619F

STUDY NUMBER(S): 353/354 R

SPONSOR: Sandoz Corp.

TESTING FACILITY: Sandoz Agrotoxicology

TITLE OF REPORT: SAN 619F: 13-Week Feeding Study in Rats

AUTHOR(S): C. Skinner

REPORT ISSUED: April 1986

CONCLUSIONS: Male and female Han Wistar rats were administered Cyproconazole in the diet at levels of 20, 80 and 320 ppm for 13 weeks; the treatment period was followed by a 4-week recovery period during which additional control and high-dose groups were fed the control diet. Changes associated with treatment, which were observed in rats administered the highest dietary level, include inhibited body weight gain, increased blood levels of creatinine and sodium with a concomitant decrease in calcium, increased liver weights and histological changes in liver. The noted changes in creatinine and calcium were also consistently observed in rats receiving the 20 ppm level but not in those administered 80 ppm. However, since these changes were not seen in treated rats after the recovery period, they should be considered treatment-related effects. A NOEL was not attained, therefore this study is not acceptable for regulatory purposes.

Classification: core-minimum (but NOEL not attained)

Quality assurance statement: Signed and dated

TEST MATERIAL

Concentration of technical material = 95.7%; Lot no.: 8405; Code name: SAN 619 F; Isomer composition: 619 A = 54.3%, 619 B = 41.4%; Description: white powder.

TEST ANIMAL DATA

Rats--Han Wistar; Age at initiation of study = 7 weeks; Body weights at study initiation: males = 176 ± 2.6g; females = 149 ± 1.3g; Identification: cage cards and individual ear marks.

Group assignment: animals were randomly selected and 15 rats/sex were assigned to 1 control and 3 dosage groups for the 90-day dosage period. Also, 15 rats/sex were assigned to an additional control or high-dose group for a 4-week recovery study.

Acclimation: 1 week

Housing: Individually in Macrolon® cages (size 3) with wood chips

Food: Kliba, powdered diet no. 21-343-4: ad libitum

Water: municipal tap water in polypropylene bottles: ad libitum

Environmental parameters: Light - 12 hr light/dark cycle; Temperature - 23 ± 2°C; Relative humidity - 50 ± 20%.

METHODS

Administration of test material

The test material was mixed with the diet from a 1% premix at levels of 20, 80 or 320 ppm which was fed to the test animals for 13 weeks; the recovery groups (1 control and 1 high-dose) were maintained on untreated diet for 4 weeks following the treatment period. Test diet mixtures were prepared weekly.

Test Diet Analysis

Cyproconazole levels in the premix and final diets were analysed prior to study initiation and at monthly intervals during the study by the registrant.

Results: The mean concentrations of Cyproconazole (4 analyses) were:

Dose(ppm)	Mean concentration(ppm)	Percent of nominal concentration
20	20.1	100.6
80	67.8	84.7
320	287.5	89.8

Symptoms and Mortality

The investigator indicated that examinations included daily inspection of skin,

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fur, feces, urine, eyes, ocular mucous membranes, respiration, circulatory and neurological activity.

Results: There were no mortalities in the study. The only clinical symptom noted in dosed animals by the investigator was piloerection, which was observed in males only (8/15 mid-dose and 14/30 high-dose).

Body Weight

Measured on day 1 and weekly thereafter.

Results: Body weight gain was slightly inhibited in high-dose males and mid- and high-dose females, however, the body weight deficits between high-dose males and females and the respective concurrent control values at the end of the treatment period were only -4.5 and -6.0%, respectively.

Body weight gain(g): 0-13 weeks

Dose(ppm)	0	20	80	320
Males	142.5	139.9	138.5	131.6
Rel.to Cont.	---	-1.8%	-2.8%	-7.6%
Females	66.0	67.6	61.1	59.2
Rel.to Cont.	---	+2.4%	-7.4%	-10.3%

Food Consumption

Determined weekly. Wasted food was salvaged and weighed.

Results: Only slight decreases in food consumption were noted for high-dose males and mid- and high-dose females.

Compound Consumption

Dietary intakes (mg/kg/day) were calculated by the investigator from body weight, food consumption and nominal dietary concentrations of test material.

Results: Adjustments to the investigator's calculated compound intake values, based on analytical data rather than nominal compound levels, gave the following mean dosage levels.

Mean intake(mg/kg/day)

Nominal dose (ppm)	20	80	320
Males	1.5	5.4	21.4
Females	1.9	5.9	27.9

Laboratory Investigations

Hematology and blood chemistry parameters were investigated in 10 males and 10 females per group at weeks 4, 8 and 13 during the treatment period and at weeks 14 and 18 during the recovery period. Animals were selected by randomized numbers generated by a computer. The rats were fasted for 16-18 hours prior to collection of blood which was drawn from the sublingual vein.

Hematology parameters

Hematocrit-Hct
Hemoglobin-Hgb
Erythrocytes-RBC
Mean cell volume-MCV
Mean corpuscular hemoglobin-MCH
Mean corpuscular hemoglobin concentration-MCHC
Leukocytes (total and differential)-WBC
Platelets
Reticulocytes

Results: A statistically significant decrease in the hematocrit with a concomitant increase in the MCHC were observed in mid- and high-dose males at weeks 4 and 8. These changes do not appear to be biologically significant since they were observed in one sex at 4 and 8 weeks only. Other changes observed during the treatment and recovery periods were sporadic and did not appear to be related to dosing.

Hematocrit and Mean Corpuscular Hemoglobin Concentration in Male Rats

Dose(ppm)	Hct(%)			MCHC(%)		
Weeks--	4	8	13	4	8	13
	Mean/S.D.					
0	48.52	50.08	48.91	35.64	35.34	36.00
	1.51	1.17	1.16	0.67	0.69	0.56
20	48.68	51.54	46.82*	35.58	34.59	36.08
	1.92	3.66	1.74	0.34	2.25	0.35
80	46.48*	45.70**	48.00	36.51**	37.65**	36.15
	1.53	1.90	1.49	0.27	0.62	0.49
320	45.84**	46.08**	48.25	36.68**	37.50**	36.35
	1.42	2.12	1.03	0.51	1.36	0.49

*$p<0.05$; **$p<0.01$ (Dunnett's t test)

Clinical chemistry parameters

Hemolytic score
Glucose
Urea
Bilirubin
Albumin
Total protein
Sodium
Potassium
Calcium
Chloride
Total cholesterol
Glutamic pyruvic transaminase(SGPT)-ALT
Glutamic oxaloacetic transaminase(SGOT)-AST
Alkaline phosphatase
Creatinine

Results: Apparent treatment-associated changes in sodium, creatinine and calcium

levels were observed. Sodium levels were decreased slightly in mid- and high-dose males ($p<0.05$) at week 4 but were elevated in all dosed males ($p<0.01$) and high-dose females ($p<0.05$) at week 13. Creatinine was increased in low- and high-dose females ($p<0.01$) at all intervals during the treatment period and in low and high-dose males at weeks 8 ($p<0.01$) and 13 ($p<0.01$ and 0.05 for low- and highdose, respectively). Calcium levels were depressed in low- and high-dose females at week 4 ($p<0.05$), low-dose males ($p<0.01$) and females ($p<0.05$) at week 8 and in low- ($p<0.01$) and high-dose ($p<0.05$) males and mid- ($p<0.05$) and high-dose ($p<0.01$) females at week 13.

The noted changes in creatinine and calcium occurred consistently in low and high-dose animals of both sexes, but rarely in mid-dose animals. The investigator did not offer a possible explanation for this observation.

During the recovery period, elevated levels of sodium in males and females ($p<0.01$) and creatinine in males ($P<0.05$) were observed at week 14 but not at week 18.

Changes in Sodium, Creatinine and Calcium levels

Sodium (MMol/L)

Mean/S.D.

Males	Treatment Period			Recovery Period	
Weeks-- / Dose (ppm)	4	8	13	14	18
0	140.2 1.9	144.4 1.2	144.9 1.7	143.4 1.3	145.5 1.0
20	141.0 1.2	145.0 1.2	147.4** 1.1	--	--
80	138.4* 1.0	145.2 1.1	147.6** 1.1	--	--
320	138.7* 0.9	145.6 1.8	147.0** 1.4	149.2** 1.5	145.9 1.1
Females					
0	145.1 1.6	143.9 2.0	142.1 0.7	143.7 0.8	145.1 1.9
20	146.3 2.1	143.9 1.8	142.4 1.5	--	--
80	146.0 1.5	145.3 0.7	143.4 0.8	--	--
320	147.1 1.5	145.6 2.1	144.1* 2.2	151.9** 1.7	145.4 1.2

*$p<0.05$; **$p<0.01$ (Dunnett's t test)

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Creatinine(Mg/Dl) Mean/S.D.

Dose (ppm)	Treatment Period Week-- 4	8	13	Recovery Period 14	18
Males					
0	0.872	0.700	0.795	0.732	0.846
	0.508	0.042	0.066	0.091	0.099
20	0.937†	1.016††	1.015††	--	--
	0.201	0.123	0.165		
80	0.741	0.718	0.835	--	--
	0.083	0.102	0.074		
320	0.854	0.904††	0.950†	0.847*	0.798
	0.080	0.133	0.104	0.105	0.078
Females					
0	0.785	0.711	0.809	1.131	1.212
	0.090	0.082	0.113	0.165	0.184
20	1.006**	0.935††	1.017**	--	--
	0.138	0.118	0.064		
80	0.842	0.761	0.842	--	--
	0.090	0.058	0.087		
320	1.035**	0.990††	1.063**	1.126	1.109
	0.056	0.150	0.142	0.088	0.149

Calcium(Mg/Dl)

Males					
0	9.46	9.33	10.71	9.07	8.39
	0.22	0.27	0.25	0.17	0.63
20	9.78	8.30**	10.12**	--	--
	0.24	0.52	0.40		
80	9.60	10.17**	10.87	--	--
	0.20	0.45	0.25		
320	9.41	9.13	10.33*	9.04	7.86*
	0.25	0.52	0.43	0.15	0.33
Females					
0	9.73	10.48	10.70	8.96	6.44
	0.19	0.17	0.31	0.29	0.68
20	9.25†	10.06*	10.44	--	--
	0.41	0.22	0.23		
80	9.44	10.47	10.30*	--	--
	0.36	0.33	0.27		
320	9.33†	10.48	10.23**	8.98	6.56
	0.38	0.43	0.36	0.25	0.94

*p<0.05; **p<0.01 (Dunnett's t test)

†p<0.05; ††p<0.01 (non-parametric--Kruskal-Wallis test)

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Urinalysis parameters

Volume		Ketones
Specific gravity		Occult blood
pH		Urobilinogen
Protein		Bilirubin
Sediment:		Glucose
RBC	Triphosate	
WBC	Amorphous urate	
Epithelial cells	Uric acid	
Calcium oxalate		

Results: No treatment-related changes were evident.

POSTMORTEM EXAMINATIONS

Organ Weights

The following organs from every animal were weighed at the end of the treatment period:

Kidneys	Ovaries
Spleen	Testes
Liver	Brain
Heart	Adrenals

Results: Although not always statistically significant, the following measurements of liver weight were higher than respective control values in high-dose males and females:

a/ Percent body weight

$p<0.01$ for males and females

b/ Percent brain weight

$p<0.01$ for females; not statistically significant for males

c/ Absolute weight

$p<0.05$ for females; not statistically significant for males

Elevated liver weights were not observed in animals examined after the recovery period.

Liver Weights in Males and Females (grams)

Dose (ppm)	Absolute Wt.	% Body Wt.	% Brain Wt.
Males		Mean/S.D.	
0	11.6	2.86	570.1
	1.7	0.29	68.0
20	11.6	2.82	579.6
	1.4	0.20	71.9
80	12.2	2.94	591.0
	1.4	0.31	73.4
320	12.4	3.18††	612.6
	2.0	0.27	87.8
Females			
0	7.2	3.05	374.6
	0.5	0.21	32.7
20	7.3	3.14	392.1
	0.6	0.30	32.7
80	7.2	3.17	391.6
	1.1	0.32	45.1
320	8.0*	3.60††	432.9††
	0.7	0.28	30.3

* $p<0.05$: Dunnett's t test
†† $p<0.01$: non-parametric, Kruskal-Wallis test

Gross Necropsy

The gross examination performed at necropsy included external surfaces, all orifices, the cranial cavity, carcass; the brain, thoracic, abdominal and pelvic cavities, with associated organs and tissues, and the neck with associated tissues.

Results: No apparent treatment-related lesions were observed.

Histological Examination

The following tissues and organs from animals in every group were fixed in 4.0% formaldehyde for histopathologic processing:

All major lesions
Brain
Pituitary
Thyroids
Heart
Liver
Kidneys
Adrenals
Prostate
Seminal Vesicles
Testes with Epididymis
Spleen
Pancreas
Parathyroid
Urinary Bladder
Stomach (2 parts)
Small Intestine
Large Intestine
Lymph Nodes (cervical, mesenteric)
Uterus
Skeletal Muscle
Skin

Ovaries
Eyes (with optical nerve)
Tongue
Trachea
Esophagus
Salivary Glands
Thymus
Lungs
Sternum (with bone marrow)
Sciatic Nerve
Spinal Cord
Arteries
Aorta (thoracic)

Results: The only histologic changes that appeared to be associated with treatment were observed in the liver. Vacuolated hepatocytes that were predominantly centrilobular were observed in 1/15 mid-dose males and 6/15 high-dose males, but not in any other group. A distinct lobular pattern, associated with enlarged hepatocytes, was observed in 5/15 males and 4/15 females, all at the high-dosage level. This change was also observed in 1/15 control males and 2/15 mid-dose males. Other hepatic changes such as vacuolated hepatocytes, which were predominantly peripherolobular, and mononuclear focal mild hepatitis occurred at frequencies that were neither treatment- nor dosage related.

The only noteworthy change observed in kidneys was tubular calcification at the coritico-medullary junction which was observed in females with comparable frequency and degree in all groups.

The noted histologic changes in liver that were prevalent in high-dose rats were not observed in rats examined after the recovery period. However, the other noted changes in liver as well as kidney, which were observed with comparable frequency in all groups, were also seen in both control and dosed rats after the recovery period.

STATISTICAL ANALYSIS

The parameters evaluated by statistical analysis included body weights, food consumption, hematology, clinical chemistry, urinalysis and organ weights. The statistical methods used in this study are described on appended page 1.

CONCLUSIONS

The changes that occurred in rats receiving the 320 ppm dosage level of Cyproconazole, which appear to treatment-related, included inhibition of body weight gain, increased blood levels of sodium and creatinine with a concomitant decrease in calcium, increased liver weights and histological changes in liver. The noted changes in creatinine and calcium were also consistently observed in rats receiving the 20 ppm level but not in those at the mid-dose (80 ppm) level.

The inhibition of body weight gain was marginal at the high-dose level, however, it occurred in both sexes. The differences in mean terminal body weights between the control and high-dose groups were less than 10% for both sexes.

The observed changes in blood creatinine and calcium levels were detected in both sexes at the 8 and 13 week intervals, while increased sodium levels were seen at week 13 only. The investigator did not offer a possible explanation for the consistent changes seen in creatinine and calcium levels in the low- and high-dose group , but not the mid-dose group. Since these changes were not observed during the recovery period, they should be considered treatment-related effects.

The histological changes observed in liver, which occurred predominantly in high-dose ra s, are apparently related to treatment.

Since treatment-related changes were observed at the lowest dietary level of Cyproconazole administered (20 ppm), a NOEL was not attained in this study. Therefore, this study is not acceptable for regulatory purposes.

Core classification: minimum

13. STATISTICAL METHODS

A computer program automatically subjected all measured values to a parametric or nonparametric statistical analysis. The particular analysis chosen depended on the distribution of the values.

a) Parametric Analysis

The program tested the homogeneity of the variances (1) then proceeded to the nonparametric analysis if the variances were not homogeneous. If the variances were homogeneous, the program performed a one-way analysis of variance (2), followed by a multiple comparison to controls using the Dunnett test (3). This test was performed only if the analysis of variance showed a significant difference between the groups. If, however, there was only one treated group, the t-test (6) was performed.

b) Nonparametric Analysis

The Kruskal-Wallis test (5) was used when more than one treated group was present. If a significant difference existed between the groups, the program performed a multiple comparison to the control group using the Dunn-Bonferroni test (4). When only one treated group existed the Mann-Whitney-U test (6) was performed for samples of four or more animals per group; with three or fewer animals per group the t-test was used.

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6. Sachs, L.: Statistische Auswertungsmethoden. Springer-Verlag, Berlin (1972).

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

CASWELL FILE

MAY 7 1990

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Cyproconazole (SAN 619F): Request by Sandoz Crop Protection Corporation to Establish a NOEL in a 13-week Feeding Study in Rats and to Accept the Study for Regulatory Purposes.

FROM: Jess Rowland, Toxicologist Jess Rowland 5/2/90
Section II, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

TO: Susan lewis/Carl Grable
Product Manager (21)
Registration Division

THRU: K.Clark Swentzel, Section Head K. Clark Swentzel 5/3/90
Section II, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)
and
Marcia van Gemert, Ph.D., Chief
Toxicology Branch II (HFAS) Marcia van Gemert 5/3/90
Health Effects Division (H7509C)

STUDY IDENTIFICATION: SAN 619F: 13-Week Feeding Study in Rats (MRID No.406077-18).HED Project No.0-0136

ACTION REQUESTED: Review and comment on response.

REGISTRANT'S REQUEST: Sandoz Crop Protection Corporation (via 10/18/89 letter from D.M.Hillebold to S.Lewis) requests establishment of a no-observed-effect level (NOEL) for SAN 619F in a 13-week feeding study in rats and reconsider the Agency's decision to not accept the study for regulatory purposes. The establishment of a NOEL is based on their argument that the changes seen in blood creatinine, sodium and calcium levels at the lowest dose tested (20 ppm) may have been fortuitous and not compound induced because similar changes in these parameters were not seen in male or female rats fed 20 or 50 ppm 619F diets in a chronic/oncogenic feeding study. Therefore, they concluded that a NOEL of 20 ppm was attained in the 13-week feeding study and consequently the study can be acceptable for regulatory purposes.

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BACKGROUND: In a subchronic toxicity study, SAN 619F was administered in the diet to groups of 15 male and 15 female HAN-Wistar rats at 0, 20, 80, or 320 ppm for 13 weeks. Toxicology Branch II (TBII) classified this study as core-minimum but not acceptable for regulatory purposes because a NOEL was not attained (Memorandum K.Swentzel, HED to L.Rossi, RD, January 19, 1989). TB II noted increased blood levels of creatine and sodium with a concomitant decrease in calcium levels in treated rats (Table 1) . The noted changes in creatinine and calcium levels were also consistently observed in rats fed the 20 and 320 ppm diets but not in those administered 80 ppm. However, since these changes were not seen in treated rats fed 320 ppm after a 4-week recovery period, these changes were considered treatment-related effects. Therefore, a NOEL was not attained and this study was classified as core-minimum but not acceptable for regulatory purposes.

RESPONSE: TB II concurs with the registrant in establishing a NOEL of 20 ppm for the 13-week study. The NOEL is established based on: 1) lack of a dose-response relationship; 2) lack of correlation with histopathological or organ weight changes; 3) because similar changes were not seen in male and female rats fed the same level (20 ppm) of SAN 619F in a chronic/oncogenicity study (MRID No.411647-01); and 4) because the creatinine, sodium, and calcium values observed in the 13-week study were within the range of baseline values for these parameters in several strains of rats of this age (Table 2). Since a NOEL was attained and a chronic/oncogenicity is available, the 13-week study can be used for regulatory purposes.

CONCLUSION: The 13-week feeding study is acceptable for regulatory purposes since a NOEL was attained.

CORE-CLASSIFICATION: Minimum; satisfies the data requirement for Guideline 82-1.

Table 1. Creatinine, Sodium and Calcium Levels in the 13-Week Feeding Study with SAN 619F.

Creatinine(Mg/Dl) Mean/S.D.

Males	Treatment Period			Recovery Period	
Week--	4	8	13	14	18
Dose (ppm)					
0	0.872	0.700	0.795	0.732	0.846
	0.508	0.042	0.066	0.091	0.099
20	0.937†	1.016††	1.015††	--	--
	0.201	0.123	0.165		
80	0.741	0.718	0.835	--	--
	0.083	0.102	0.074		
320	0.854	0.904††	0.950†	0.847*	0.798
	0.080	0.133	0.104	0.105	0.078
Females					
0	0.785	0.711	0.809	1.131	1.212
	0.090	0.082	0.113	0.165	0.184
20	1.006**	0.935††	1.017**	--	--
	0.138	0.118	0.064		
80	0.842	0.761	0.842	--	--
	0.090	0.058	0.087		
320	1.035**	0.990††	1.063**	1.126	1.109
	0.056	0.150	0.142	0.088	0.149

Sodium (MMol/L) Mean/S.D.

Males	Treatment Period			Recovery Period	
Weeks--	4	8	13	14	18
Dose (ppm)					
0	140.2	144.4	144.9	143.4	145.5
	1.9	1.2	1.7	1.3	1.0
20	141.0	145.0	147.4**	--	--
	1.2	1.2	1.1		
80	138.4*	145.2	147.6**	--	--
	1.0	1.1	1.1		
320	138.7*	145.6	147.0**	149.2**	145.9
	0.9	1.8	1.4	1.5	1.1
Females					
0	145.1	143.9	142.1	143.7	145.1
	1.6	2.0	0.7	0.8	1.9
20	146.3	143.9	142.4	--	--
	2.1	1.8	1.5		
80	146.0	145.3	143.4	--	--
	1.5	0.7	0.8		
320	147.1	145.6	144.1*	151.9**	145.4
	1.5	2.1	2.2	1.7	1.2

Calcium(Mg/Dl)

Males					
0	9.46	9.33	10.71	9.07	8.39
	0.22	0.27	0.25	0.17	0.63
20	9.78	8.30**	10.12**	--	--
	0.24	0.52	0.40		
80	9.60	10.17**	10.87	--	--
	0.20	0.45	0.25		
320	9.41	9.13	10.33*	9.04	7.86*
	0.25	0.52	0.43	0.15	0.33
Females					
0	9.73	10.48	10.70	8.96	6.44
	0.19	0.17	0.31	0.29	0.68
20	9.25†	10.06*	10.44	--	--
	0.41	0.22	0.23		
80	9.44	10.47	10.30*	--	--
	0.36	0.33	0.27		
320	9.33†	10.48	10.23**	8.98	6.56
	0.38	0.43	0.36	0.25	0.94

*p<0.05; **p<0.01 (Dunnett's t test)

†p<0.05; ††p<0.01 (non-parametric--Kruskal-Wallis test)

Table 2. Baseline Values for Creatinine, Sodium and Calcium in Several Strains of Male Rats (11 to 21 weeks of Age).[a]

Strain	Creatinine (mg/dl)		Sodium (mg/l)		Calcium (mg/l)	
	Mean	Range	Mean	Range	Mean	Range
Charles River	1.0	0.6-1.4	147	144-150	11	9.9-12.1
Harlan S-D	0.5	0.4-0.6	153	150-160	11.3	10.8-12.1
Fisher 344	0.5	0.4-0.5	150	147-153	11.2	10.5-11.7
Holtzman	0.5	0.4-0.6	152	151-155	10.4	9.7-11.7

[a] SOURCE: Charles River Breeding Laboratories. Baseline Hematology Clinical Chemistry Values for Charles River CD [Crl:CD(SD)BR] Rats as a Function of Sex and Age. Technical Bulletin. Volume 3, No.3, 1984.

Harlan Sprauge Dawley Inc. Serum Chemistry/Complete Blood Counts-Rats. Final Report. 1989.

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Reviewed by: K. Clark Swentzel K. Clark Swentzel 10/20/88 007003
Section 2 , Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. James N. Rowe 11/16/88
Section 2 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 13-Week Feeding Study TOX. CHEM. NO.: 272E

MRID NO.: 406077-19

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol

SYNONYMS: Cyproconazole; SAN 619F

STUDY NUMBER(S): 6521/86

SPONSOR: Sandoz Corp.

TESTING FACILITY: Research and Consulting Company, A.G., Itingen, Switzerland

TITLE OF REPORT: 13-Week Feeding Study in Beagle Dogs

AUTHOR(S): S. Warren, S. Carpy, C. Skinner & J. Karapally

REPORT ISSUED: December 12, 1986

TEST DATES: April 17, 1985 - July 23 & 24, 1985

CONCLUSIONS

Male and female beagle dogs were administered Cyproconazole in the diet at levels of 20, 100 and 500 ppm for 13 weeks. Changes associated with treatment, observed in both sexes administered the highest dietary level, included "slack muscle tone", inhibited body weight gain, increased platelet counts, decreased: bilirubin, total cholesterol, HDL-cholesterol, triglycerides, total protein and albumin and increased alkaline phosphatase and gamma glutamyl transferase; decreased food consumption was seen in high-dose males. Increased absolute and relative liver weights and increased relative kidney weights were noted for high-dose males and females; relative brain weights were increased in high-dose females. Histopathologic evidence of liver toxicity in high-dose males and females included hepatocytomegaly, degeneration of single hepatocytes and cytoplasmic inclusions. Evidence of liver toxicity in mid-dose dogs was increased absolute liver weights in males and hepatocytomegaly in males and females.

The LEL in this study, based on adverse effects in liver, was 100 ppm (approximately 4 mg/kg/day and the NOEL was 20 ppm (approximately 0.8 mg/kg/day).

Since this study was not inspected by a QAU during the in-life phase, a data audit, signed and dated by a QA Officer, must be submitted to the Agency before this study can be accepted for regulatory purposes.

Core classification: supplementary (can be upgraded to minimum provided an acceptable QAU audit is provided as noted above)

Quality assurance statement: Signed and dated by the Study Director (NOT ACCEPTABLE)

TEST MATERIAL

Concentration of technical material = 95.6%; Lot no.: 8507; Code name: SAN 619 F; Isomer composition: not reported; Description: white powder.

TEST ANIMAL DATA

Dogs--Beagle; Age at initiation of study = 7 months; Body weights at study initiation: males = 9.8 ± 0.8 kg; females = 8.5 ± 0.9 kg; Identification: individual numbers.

Group assignment: animals were randomly selected and 4 dogs/sex were assigned to 1 control and 3 dosage groups for the 13-week dosage period.

Acclimation: 8 weeks

Housing: Individually in hanging steel mesh self-cleaning cages

Food: Kliba, powdered diet no. 24-335-1: 500g provided per day

Water: municipal tap water: ad libitum

Environmental parameters: Light - 12 hr light/dark cycle; Temperature - 22 ± 2°C; Relative humidity - 55 ± 10%.

METHODS

Administration of test material

The test material was mixed with the diet from a 1% premix at levels of 20, 100 or 500 ppm which was fed to the test animals for 13 weeks. The premix was prepared monthly and stored at 3-5°C. The frequency of diet preparation was not indicated.

Test Diet Analysis

Cyproconazole levels in the premix and final diets were analysed prior to study initiation and subsequently at monthly intervals by the registrant.

Results: The mean concentrations of Cyproconazole (Weeks: 1,4,8 & 13) were within acceptable limits (± 15% of nominal).

Dose(ppm)	Mean concentration(ppm)	Percent of nominal concentration
20	20.9	104.5
100	92.8	92.8
500	457.5	91.5

Symptoms and Mortality

The dogs were examined twice daily for treatment-induced symptoms. The examinations included oral, behavioral, color, feces and urine inspections.

Results: All dogs survived until the scheduled terminal sacrifice. The investigator indicated that "slack muscle tone" was observed in all high-dose animals. This change was first observed in week 1 and all dogs were affected after 2 weeks of treatment. This effect persisted until the end of the study in 3/4 males and 2/4 females. No other relevant clinical changes were noted.

Ophthalmoscopy

Control and high-dose animals were examined prior to the commencement of treatment and prior to termination using a fundus camera following administration of a mydriatic.

Results: No lesions were detected.

Body Weights

Individual body weights were recorded weekly.

Results: Body weight gain was inhibited in high-dose males and females. Mean terminal body weights of dogs in this group were -12.8 and -10.5% for males and females, respectively, relative to corresponding control weights.

Body weight gain

Interval(weeks)

Dose(ppm)	0	20	100	500
Males	Kg/% difference(rel. to control)			
0 - 6	1.4	1.4	1.6	0.5
	---	0.0	+14.3	-64.3
6 - 13	0.7	1.0	0.8	0.9
	---	+42.9	+14.3	+28.6
0 - 13	2.1	2.4	2.4	1.4
	---	+14.3	+14.3	-33.3
Females				
0 - 6	0.7	1.0	0.7	0.3*
	---	+42.9	0.0	-57.1
6 - 13	0.5	0.9	0.6	0.2
	---	+80.0	+20.0	-60.0
0 - 13	1.2	1.9*	1.3	0.5*
	---	+58.3	+8.3	-58.3

* $p<0.05$ (no statistical analysis performed on data tabulated for weeks 6 - 13)

Food Consumption

Determined twice weekly and expressed as grams of food consumed per dog per day.

Results: Food consumption was slightly lower in high-dose males throughout the study. During the first 6 weeks, consumption was 19% lower and during the last 7 weeks, 11% lower, in comparison to controls. Food consumption values in the other treatment groups were comparable to respective control values for both males and females.

Calculated Intake of SAN 619 F

Individual compound consumption values were calculated at weekly intervals from individual bodyweight and food consumption data and nominal dietary concentrations of SAN 619 F.

Results: The investigator calculated the following mean compound consumption values for each group:

SAN 619 F intake values

Dose (ppm)	Males		Females	
	mean	max.	mean	max.
	(mg/kg/day)			
20	0.77	0.95	0.70	0.83
100	4.00	4.59	3.25	3.96
500	18.18	19.76	19.17	21.95

Laboratory Investigations

Blood profiles and clinical chemistry values were determined for all dogs. The animals were fasted for approximately 16 hours prior to collection of venous blood samples. Hematology parameters were measured at weeks 0, 4, 8 and 13. Clinical chemistry determinations were made at weeks -1, 0, 4, 8, 12 and 13, unless indicated otherwise. These determinations included all of the parameters stipulated in the Subdivision F Guidelines.

Hematology parameters

Hematocrit-Hct
Hemoglobin-Hgb
Erythrocytes-RBC
Mean cell volume-MCV
Mean corpuscular hemoglobin-MCH
Mean corpuscular hemoglobin concentration-MCHC
Leukocytes (total and differential)-WBC
Platelets
Reticulocytes

Results: Increased platelet counts were observed in high-dose males and females only. These counts exceeded control as well as baseline values at all post-treatment intervals.

Platelet counts (high-dose vs. control)

Dose (ppm) / Week--	0	4	8	13
		Mean/S.D. ($10^3/mm^3$)		
Males				
0	299.5	292.5	292.5	253.5
	56.0	78.6	85.0	57.0
20	282.3	306.8	279.3	267.3
	95.6	43.3	14.4	22.1
100	351.8	358.0	329.3	296.0
	36.7	43.1	50.2	42.2
500	333.5	410.8	429.0	442.3**
	37.0	72.7	110.2	116.5
Females				
0	348.8	324.3	316.8	310.0
	111.7	94.3	86.9	83.5
20	284.3	276.8	276.3	284.3
	93.3	71.9	82.0	87.3
100	279.5	287.5	279.5	275.0
	53.6	32.3	54.8	47.2
500	334.3	437.5	456.5*	427.5
	78.0	61.9	58.3	66.6

* $p<0.05$; ** $p<0.01$ (relative to controls)

Clinical chemistry parameters

	Test Interval (weeks)				
	-1	0	4	8	13
Hemolytic score	x	x	x	x	x
Glucose	x	x	x	x	x
Urea	x	x	x	x	x
Total Bilirubin	x	x	x	x	x
Albumin	x	x	x	x	x
Total protein	x	x	x	x	x
Sodium	x	x	x	x	x
Potassium	x	x	x	x	x
Calcium	x	x	x	x	x
Chloride	x	x	x	x	x
Total Cholesterol				x	x
High Density Lipoprotein-Cholesterol(HDL-Chol)				x	x
Triglycerides		x	x	x	x
Glutamic pyruvic transaminase(SGPT)-ALT	x	x	x	x	x
Glutamic oxaloacetic transaminase(SGOT)-AST	x	x	x	x	x
Gamma glutamyl transferase(GGT)	x	x			x
Glutamate dehydrogenase(GLDH)	x	x			x
Leucine aryl amidase(LAP)	x	x			x
Alkaline phosphatase	x	x	x	x	x
Creatinine	x	x	x	x	x
Lactate dehydrogenase(LDH)	x	x	x	x	x
Inorganic Phosphorous	x	x	x	x	x
Creatinine phosphokinase(CPK)				x	x
Cortisol					x
Testosterone					x

Results:

Statistically significant differences in treatment-related changes, in comparison to controls and/or baseline data, are indicated in the following tabulated data as follows: 1) comparison to controls (* $p<0.05$, ** $p<0.01$ - parametric test; † $p<0.05$, †† $p<0.01$ - non-parametric test); 2) comparison to baseline ($ $p<0.05$, $$ $p<0.01$ - parametric test).

Changes in clinical chemistry parameters observed in high-dose dogs, which appeared to be treatment-related, included decreases in bilirubin, total cholesterol, HDL-cholesterol, triglycerides, total protein and albumin and increases in alkaline phosphatase and GGT. Other fluctuations noted in high-dose animals, which did not appear to be related to treatment because they were not dose-related and/or were comparable to baseline values, included increases in creatinine, ALT, CPK, GLDH, cortisol and testosterone as well as decreases in calcium and inorganic phosphorous.

Although not always statistically significant, the noted treatment-related changes were observed in both sexes at the 4, 8 and 13 week intervals with the exception of decreased cholesterol (total and HDL), observed at weeks 8 and 13 (only intervals for measurement) and increased GGT at week 13 (measured at weeks -1, 0 and 13).

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Treatment-related changes in clinical chemistry parameters

Dose(ppm) / Weeks--	-1	0	4	8	13
			Mean/S.D.		
Bilirubin(uMol/L)					
Males					
0	0.428 0.375	1.323 0.106	0.818 0.401	1.145 0.232	1.708 0.308
20	1.207 0.339	1.340 0.155	1.018 0.362	1.233 0.372	1.640 0.252
100	0.828 0.577	1.048 0.249	0.825 0.567	1.045 0.235	1.635 0.267
500	1.205 0.512	1.250 0.225	0.575 0.443	0.785^{S} 0.395	1.248 0.415
Females					
0	0.548 0.409	1.255 0.385	1.035 0.826	1.155 0.394	1.625 0.249
20	0.993 0.574	1.733 0.655	1.250 0.494	1.453 0.582	2.073 0.470
100	0.858 0.573	1.700 0.254	1.130 0.733	1.440 0.385	1.975 0.168
500	0.950 0.478	1.570 0.135	0.518^{S} 0.602	0.673^{S} 0.360	1.303 0.262
Total cholesterol(MMol/L)					
Males					
0	—	—	—	4.195 0.599	3.968 0.802
20	—	—	—	4.310 0.221	4.360 0.311
100	—	—	—	4.510 0.700	4.468 0.932
500	—	—	—	2.043** 0.439	2.078** 0.479
Females					
0	—	—	—	3.443 0.327	3.828 0.676
20	—	—	—	5.713* 1.604	6.153** 0.974
100	—	—	—	4.185 0.408	4.348 0.300
500	—	—	—	2.563 0.728	2.793 0.607

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Treatment-related chances in clinical chemistry parameters

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Dose(ppm) / Weeks--	-1	0	4	8	13
			Mean/S.D.		
HDL-Cholesterol(MMol/L)					
Males					
0	--	--	--	4.565 0.872	4.288 0.747
20	--	--	--	4.605 0.536	5.418 1.002
100	--	--	--	4.813 0.895	4.560 0.660
500	--	--	--	2.280** 0.544	2.908 0.389
Females					
0	--	--	--	3.648 0.334	4.638 0.591
20	--	--	--	6.343** 1.486	5.223 1.639
100	--	--	--	4.468 0.640	3.245 1.591
500	--	--	--	2.820 0.722	2.568 0.527
Triclycerides(MMol/L)					
Males					
0	--	0.453 0.099	0.500 0.073	0.443 0.132	0.373 0.075
20	--	0.583 0.099	0.565 0.114	0.495 0.116	0.443 0.053
100	--	0.413 0.032	0.418 0.021	0.420 0.068	0.383 0.039
500	--	0.465 0.060	0.258† 0.022	0.313 0.040	0.300 0.048
Females					
0	--	0.378 0.019	0.485 0.095	0.478 0.085	0.400 0.048
20	--	0.568* 0.053	0.610 0.076	0.563 0.159	0.565 0.118
100	--	0.475 0.051	0.468 0.061	0.440 0.081	0.505 0.102
500	--	0.513* 0.120	0.243** 0.072	0.335 0.146	0.365 0.123

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Treatment-related changes in clinical chemistry parameters

Dose(ppm) / Weeks--	-1	0	4	8	13
			Mean/S.D.		
Total Protein(G/L)					
Males					
0	49.92 4.82	59.22 2.51	58.72 3.15	53.05 2.83	60.93$ 2.44
20	44.03 10.61	58.28 3.24	61.63 2.41	55.40 2.15	62.45$ 2.85
100	52.45 1.58	57.85 2.06	59.93 2.80	54.13 2.51	63.85$$ 1.42
500	51.23 4.18	58.35 2.34	55.25 1.41	49.90 1.28	57.42$ 3.48
Females					
0	54.00 4.30	59.63 3.32	61.07 3.53	55.15 3.16	62.20$ 2.15
20	53.30 3.11	57.92 2.40	62.38 1.76	56.05 1.60	63.80$ 2.04
100	52.75 1.92	57.57 0.69	60.38 2.82	54.55 2.85	64.22$ 3.10
500	54.80 1.25	57.78 2.98	54.85* 4.04	49.52*$ 3.67	59.27 6.90
Albumin(G/L)					
Males					
0	32.40 1.59	29.68 0.83	30.10 1.24	30.48 0.85	30.78 0.90
20	30.07 1.17	28.43 1.51	29.83 1.53	30.35 1.50	29.75 1.67
100	31.78 0.94	28.35 1.76	28.10 1.32	28.58†$ 1.33	28.78$ 0.68
500	32.10 2.07	29.38 1.18	24.58** 1.24	24.10†$$ 2.60	23.45**$$ 2.79
Females					
0	33.38 0.96	29.53 1.14	30.60 0.53	31.93$ 0.67	30.70$$ 0.24
20	33.78 2.48	29.33 1.50	31.68 1.58	33.13 1.64	31.45 2.33
100	33.30 2.50	28.58 2.41	30.58 1.85	31.10$ 1.93	29.95$ 2.12
500	32.38 1.82	28.30 1.31	24.25** 1.55	24.43**$$ 1.70	23.95†$$ 2.30

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Treatment-related changes in clinical chemistry parameters

Dose(ppm) Weeks--	-1	0	4	8	13
			Mean/S.D.		
Alkaline Phosphatase(U/L)					
Males					
0	200.00 23.51	180.50 21.08	142.25 10.05	121.50$ 21.02	88.62$$ 7.51
20	216.33 34.70	206.25 23.89	188.25† 34.65	167.00 46.31	137.67 42.00
100	191.50 12.50	188.75 24.40	151.25 26.74	132.75$ 34.07	119.25$ 33.59
500	213.25 28.43	201.25 35.08	394.75† 125.99	447.25† 175.05	459.25** 161.50
Females					
0	219.00 43.89	210.00 39.10	181.25 49.34	149.75$ 29.03	118.85$$ 25.69
20	181.75 35.80	166.75 31.14	138.50 25.65	132.05$ 42.26	99.15$$ 22.99
100	260.00 101.72	242.00 93.83	230.00 62.69	188.80$ 6861	156.05$ 54.80
500	231.50 62.94	209.75 58.70	349.00** 63.68	466.50†$ 139.85	456.00**$$ 97.83
Gamma clutamyl transaminase(U/L)					
Males					
0	2.180 0.176	0.983 0.322	--	--	2.755 0.357
20	1.557 0.630	1.323 0.202	--	--	2.238 0.384
100	1.405 0.407	1.218 0.463	--	--	2.785$$ 0.426
500	1.905 0.523	1.763 0.740	--	--	4.330†$$ 0.989
Females					
0	2.110 0.600	1.148 0.478	--	--	2.413 0.377
20	1.123 0.266	0.953 0.573	--	--	2.433$$ 0.149
100	1.793 0.207	1.273 0.633	--	--	4.085†$$ 0.816
500	1.375 0.956	1.718 0.730	--	--	4.133†$$ 0.374

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Urinalysis

Urine samples were collected over a 4 hour period at each interval (-2, 4, 8 and 13 weeks), during which time food and water were withheld. The following parameters were investigated immediately after each collection period:

Urinalysis parameters

Volume
Specific gravity
pH
Protein
Sediment:
- RBC
- WBC
- Epithelial cells
- Calcium oxalate
- Triphosate
- Amorphous urate
- Uric acid

Ketones
Occult blood
Urobilinogen
Bilirubin
Glucose

Results: There were no noted changes in the investigated urinary parameters indicative of a compound-related effect.

Organ Weights

The following organs were excised, trimmed and weighed:

Kidneys
Liver
Testes/Ovaries
Adrenals
Brain
Thyroids

The investigator indicated that organ weight to bodyweight and organ weight to brain weight ratios were calculated.

Results: It is apparent from the individual data that organ to body weight ratios were calculated, however, only summarized data for absolute organ weights in males and females and organ to body weight ratios for females were submitted. Also, calculated organ to brain weight ratios were not submitted.

Absolute liver weights were increased in mid- and high-dose males and high-dose females. Relative weights (% body weight) for brain, liver and kidneys were increased in high-dose females. Relative liver and kidney weights were also increased in males.

Liver weights

Mean/S.D.

Dose (ppm)	Males		Females	
	Absolute wt. (g)	Relative wt.† (% B.W.)	Absolute wt. (g)	Relative wt. (% B.W.)
0	307.7	2.6	261.1	2.9
	46.4	0.3	20.5	0.1
20	315.7	2.5	293.3	2.8
	38.6	0.4	16.2	0.4
100	386.6	3.3	265.2	2.8
	34.7	0.4	25.1	0.2
500	440.9**	4.3	348.4**	4.4**
	55.9	0.5	48.0	0.3

* p<0.05, ** p<0.01; † Statistical analysis not performed

Kidney weights

Mean/S.D.

Dose (ppm)	Males		Females	
	Absolute wt. (g)	Relative wt.† (% B.W.)	Absolute wt. (g)	Relative wt. (% B.W.)
0	54.27	0.46	37.89	0.43
	14.75	0.11	1.77	0.05
20	56.94	0.44	42.41	0.40
	6.84	0.05	3.43	0.01
100	56.88	0.48	39.12	0.41
	10.70	0.06	2.73	0.03
500	51.55	0.51	38.95	0.49*
	7.94	0.08	2.36	0.02

Relative brain weights (% B.W.)

	Males†	Females
0	0.70	0.90
	0.00	0.10
20	0.65	0.80
	0.06	0.10
100	0.70	0.80
	0.08	0.10
500	0.73	1.00**
	0.05	0.10

* $p<0.05$, ** $p<0.01$, † Statistical analysis not performed

Macroscopic Examination

This procedure included examination of external surfaces, all orifices, the cranial cavity, the carcass; the brain, thoracic, abdominal and pelvic cavities with associated organs and tissues as well as the neck with its tissues.

Results: No treatment-related changes were noted.

Histopathologic Examination

Representative samples of all of the following organs, tissues and anomalies from all animals were fixed in 4% formaldehyde for histopathological processing.

All major lesions
Brain
Pituitary
Thyroid
Heart
Liver
Kidneys
Adrenals
Prostate
Thymus
Testes with Epididymis
Gall Bladder
Spleen
Pancreas
Lungs
Urinary Bladder
Lymph Nodes (cervical, mesenteric)
Skin
Uterus
Skeletal Muscle
Stomach (2 parts)
Duodenum
Jejunum
Ileum

Mammary Gland
Sternum (w.bone marrow)
Cervix
Ovaries
Eyes (with optical nerve)
Tongue
Trachea
Esophagus
Salivary Glands
Cecum
Colon
Rectum
Bone Marrow (femoral)
Sciatic Nerve
Spinal Cord(cervical/thoracic)
Arteries
Aorta (thoracic)
Parathyroid

Results: The only microscopic changes that appear to be related to treatment were observed in the liver of males and females of the mid- and high-dose groups. Hepatocytomegaly occurred at a frequency of 2/4 for both mid-dose males and females and 4/4 for both high-dose males and females. Also, degeneration of single hepatocytes and cytoplasmic inclusions were each observed at a rate of 1/4 at the high-dose in both males and females. Other noted changes occurred sporadically or were observed at comparable freqeuncies between groups and could not conclusively be associated with treatment.

Statistical Analysis

Body weight, food consumption hematology, clinical chemistry, urinalysis, organ weight and organ weight/body weight ratios were analysed for differences between treated and control group mean values. Intra-group differences between pre- and post treatment mean values were also analysed, where applicable. A description of the methodology for the statistical analyses used in this study is shown on appended page 1.

Conclusions

Compound-related effects, which were evident among males and females in the high-dose group, were reflected by changes in several of the investigated parameters. During the clinical observations, the investigator indicated that these animals had "slack muscle tone" throughout the study. Although body weight gain was inhibited in both sexes, food consumption was decreased among males only. The clinical laboratory investigations revealed the following changes among high-dose males and females: increased platelet counts; decreased bilirubin, total cholesterol, HDL-cholesterol, triglycerides, total protein and albumin (primary cause of decreased total protein) and increased alkaline phosphatase and gamma glutamyl transferase(GGT). Group organ weight data showed increased absolute and relative (% body weight) liver weight and increased relative kidney weight in high-dose males and females and increased relative brain weight in high-dose females. Histopathologic data revealed liver changes which included hepatocytomegaly, degeneration of single hepatocytes and cytoplasmic inclusions.

The only apparent compound-related effects observed in mid-dose animals were changes in liver which included increased absolute liver weight in males and hepatocytomegaly in males and females.

The most obvious effects from Cyproconazole in this study were changes in the investigated parameters indicative of liver toxicity. The decreased levels of cholesterol and increased levels of alkaline phosphatase and GGT in high-dose dogs may indicate cholestatic injury. The decreased albumin in high-dose animals may also be due to liver toxicity. Additional evidence of liver toxicity were previously noted increased liver weights and morphologic changes in the liver of mid- and high-dose animals.

The LEL in this study, based on adverse effects in liver, was 100 ppm (approximately 4 mg/kg/day). The NOEL was 20 ppm (approximately 0.8 mg/kg/day).

Since the Quality Assurance statement was signed by the Study Director instead of a Quality Assurance Officer, this study is unacceptable for regulatory purposes. Since this study was not inspected by the QAU during the in-life phase, a data audit, signed and dated by a QA Officer, must be submitted to the Agency before this study can be accepted.

Core-classification: supplemental (can be upgraded to minimum provided an acceptable QAU audit is provided as noted above)

J. STATISTICAL ANALYSES

Statistical Analysis was performed on all tabulated numerical data in Appendix A, as follows:

Cortisol and Testosterone results were evaluated using Student-Neuman-Keuls test.

Organ weights and organ weight ratios were evaluated using Dunnett's test.

For all other data, a computer program automatically subjected all measured values to a parametric or nonparametric statistical analysis. The particular analysis chosen depended on the distribution of values.

a) Parametric Analysis

The program tests the homogeneity of the variances (1) then proceeds to the nonparametric analysis if the variances are not homogeneous. If the variances are homogeneous, the program performed a one-way analysis of variance (2), followed by a multiple comparison to controls using the Dunnett test (3). This test is performed only if the analysis of variance shows a significant difference between the groups. If, however, there is only one treated group, the t-test (6) is performed.

b) Nonparametric Analysis

The Kruskal-Wallis test (5) is used when more than one treated group is present. If a significant difference exists between the groups, the program performs a multiple comparison to the control group using the Dunn-Bonferroni test (4). When only one treated group exists the Mann-Whitney-U test (6) is performed for samples of four or more animals per group; with three or fewer animals per group the t-test is used.

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DATA EVALUATION REPORT

007003

STUDY TYPE: Teratogenicity Study in Rats

TOX. CHEM. NO.: 272E

MRID NO.: 406077-21

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol

SYNONYMS: Cyproconazole; SAN 619F

STUDY NUMBER(S): 048712

SPONSOR: Sandoz Corp.

TESTING FACILITY: Research and Consulting Company, A.G., Itingen, Switzerland

TITLE OF REPORT: Teratogenicity Study in Rats with SAN 619F

AUTHOR(S): C. Klotzche

REPORT ISSUED: December 11, 1985

TEST DATES: July 8, 1985 - August 14, 1985

CONCLUSIONS

A suspension of Cyproconazole in distilled water mixed with carboxymethylcellulose sodium salt (CMC, 4%) was administered daily to pregnant Wistar/Han rats (25/group) via oral gavage from day 6 through 15 of gestation at dosage levels of 6, 12, 24 and 48 mg/kg. Evidence of maternal toxicity included inhibited body weight gain during treatment at dosage levels of 12 mg/kg and above and decreased body weight and food consumption among females in the 24 and 48 mg/kg dosage groups. However, since the noted differences in maternal body weights were influenced by treatment-related intrauterine effects (e.g., increased number of resorptions, decreased fetal weight etc.), the evidence for maternal toxicity is equivocal.

Evidence of fetal toxicity was apparent from observed dose-related increases in the number of fetuses with supernumerary ribs at dosages of 6 mg/kg and above. Embryo/fetal toxicity was apparent at 24 and 48 mg/kg from the following observations: decreased total number of fetuses/dam, decreased number of live fetuses/dam, increased percentage and number of fetal resorptions, decreased body weight and incomplete ossification in phalangeal nuclei and the absence of ossification in calcanea.

There was evidence of teratogenicity in the 24 and 48 mg/kg groups. Hydrocephaly was observed in 1 fetus in the 24 mg/kg and 2 fetuses in the 48 mg/kg groups. Cleft palate was observed in 2 fetuses in the 48 mg/kg group.

The NOEL for developmental toxicity was not determined, based on induced fetotoxicity (supernumerary ribs) at 6 mg/kg. The NOEL for maternal toxicity was 6 mg/kg (equivocal).

The registrant should submit data which show the litter incidence of supernumerary ribs (number of litters/group with the noted change) with appropriate statistical analyses to aid in the determination of a possible NOEL for developmental toxicity in this study.

Core classification - supplementary (can possibly be upgraded to minimum by submitting requested data)

Quality assurance statement: Signed and dated by the QAO

RANGE-FINDING STUDY

The main teratogenicity study was preceded by a pilot study, which was performed to determine appropriate dosage levels of Cyproconazole for the main study. This study was also performed by RCC.

Study Title: Dose-Finding Teratogenicity Study in rats with SAN 619F

Study No.: 048701

Date of report: July 9, 1985

Date of study: May 19, 1985 - June 13, 1985

Author: H. Becker

Materials and Methods

Cyproconazole(Batch No. 8507, purity-95.6%) was administered to mated female Wistar KFM-Han rats (5/group) via gavage at dosage levels of 0(vehicle: distilled water with 4% carboxymethylcellulose sodium salt, 99.5%), 7.5, 30, 75 and 120 mg/kg on days 6 through 15 of gestation. The dams were sacrificed on day 21 of gestation; postmortem examinations included macroscopic inspection of internal organs and uterus (uterine contents and the position of each fetus) as well as corpora lutea counts. The fetuses were sexed, weighed, examined for external gross abnormalities and those without malformations were discarded.

Results

Maternal toxicity was evident from decreased food consumption at 7.5 mg/kg and above and inhibited body weight gain at 30 mg/kg and above, during the treatment period.

Uterine and fetal examinations revealed developmental toxicity at 30, 75 and 120 mg/kg, evident from increased rates of early resorptions (post implantation), decreased fetal body weight and increased incidences of fetuses with cleft palate (especially at 120 mg/kg).

Conclusions

The dosage levels of Cyproconazole chosen for the main teratogenicity study were 6, 12, 24 and 48 mg/kg/day. Based on the treatment-related effects observed in this study, the selected dosages appear to be appropriate.

Core classification: Supplementary data (Range-finding study)

Main Developmental Toxicity Study

TEST MATERIAL

Concentration of technical material = 95.6%; Lot no.: 8507; Code name: SAN 619 F; Isomer composition: not reported; Description: white powder.

TEST ANIMAL DATA

Rats--Wistar/Han; 125 mated females, 25/group; Age at initiation of study = 11 weeks; Body weights at study iniation (post coitum): 180 - 236g; Identification: individual cage numbers and corresponding ear tag.

Group assignment: 25 females were randomly assigned to 1 control and each of 4 dosage groups.

Acclimation: 9 days

Housing: Individually in Makrolon cages with steel mesh tops and standardized granulated softwood bedding.

Food: Pelleted Kliba 343 rat maintenance diet, ad libitum.

Water: municipal tap water: ad libitum

Environmental parameters: Light - 12 hr light/dark cycle; Temperature - 22 ± 2°C; Relative humidity - 55 ± 10%; 10-15 air changes/hr; monitored hourly.

METHODS

Mating

Following acclimation, the females were housed with males(1:1) until either the daily vaginal smear was sperm-positive or a copulation plug was observed(designated day 0 post coitum). Each mated female was housed individually.

Preparation of Test Material

The vehicle, distilled water with carboxymethylcellulose sodium salt (CMC; 4%), was added to a weighed amount of Cyproconazole; homogeneity was maintained by constant stirring. Neither the concentration of test compound nor the proportions of water and CMC were indicated.

Administration of Test Material

The test material/vehicle mixtures were prepared daily prior to administration. The test material was administered daily via oral gavage from day 6 through day 15 of gestation. The controls received vehicle only. All groups received a volume of 10 ml/kg body weight with a daily adjustment of individual volume to the actual body weight.

Test Mixture Analysis

Determination of concentration as well as the homogeneity and stability of the test mixtures were performed once during the treatment period. Samples were taken immediately after mixture preparation and again after 90 minutes.

037083

Results

The measured concentrations of Cyproconazole ranged from 83.3 to 108.3% and 83.3 to 116.7% of nominal concentrations immediately after preparation and 90 minutes after preparation, respectively(data on appended page 1).

Maternal Mortality

Animals were observed twice daily for possible mortalities.

Results: None of the females died during the study.

Signs and Symptoms

Animals were examined twice daily.

Results: No apparent treatment-related clinical signs or symptoms were observed.

Number of Dams Examined

Only dams with live fetuses on day 21 post coitum were included in the calculations of body weight gain, corrected body weight gain, mean daily food consumption and reproduction data.

Dose (mg/kg)	0	6	12	24	48
No. of Dams	23	22	23	25	22

Maternal Body Weight and Body Weight Gain

Body weights were measured daily from day 0 until day 21 post coitum. Calculations of body weight gain during the treatment period started on day 6 post coitum (immediately prior to the first administration) and ended on day 16 post coitum (approximately 24 hr after the last administration).

Results: Mean body weights were significantly lower ($p<0.05$, Dunnett's t test) than corresponding control values at 24 mg/kg during gestation days 12 - 21 and at 48 mg/kg during gestation days 8 and 12 - 21 (tabulated on appended pages 2 - 11).

Mean body weight gain during treatment (gestation days 6 - 16) was decreased (>10% lower than control value) in the 12, 24 and 48 mg/kg groups. Inhibited body weight gain was still evident after treatment (days 16-21) in the 24 and 48 mg/kg groups, however, the differences from the control value were less than those noted during the treatment period. Body weight gain before treatment (0-6 days) was comparable between all groups (8.1 - 8.8%).

Mean maternal body weight gain during treatment

Dosage(mg/kg)	0	6	12	24	48
B.W. gain(g)	44	42	39	35	33
% increase	19.6	18.7	17.2	15.7	14.7
Diff. from controls (%)	---	-4.8	-11.4	-20.5	-25.0

Mean maternal body weight gain after treatment

Dosage(mg/kg)	0	6	12	24	48
B.W. gain(g)	48	51	53	43	42
% increase	17.8	19.1	19.9	16.7	16.3
Diff. from controls (%)	---	+6.3	+10.4	-10.4	-12.5

Corrected Body Weight Gain

Corrected body weight gain was calculated as follows:

[Body weight on gestation day 21 - Body weight on gestation day 6 - Uterus weight at necropsy on gestation day 21]

Results: Corrected body weight gains were comparable between treated and control groups. Therefore, the noted decreases in body weight and body weight gain among dams in the treatment groups were apparently influenced by increased resorptions and/or decreased fetal weights (see fetal data below).

Mean corrected body weight gain

Dose (mg/kg)	0	6	12	24	48
% gain rel. to day 6	9.5	8.4	7.2	9.3	9.5

Food Consumption

Food consumption was determined on days 6, 11, 16 and 21 of gestation.

Results: Food consumption was decreased in all treated groups during the treatment period; these decreases were statistically significant in the 24 and 48 mg/kg dosage groups.

Mean daily food consumption

Dose (mg/kg)	Gestation Days		
	0 - 6	6 - 16	16 - 21
	[g/day (% diff. from contr.)]		
0	18.7	21.7	23.2
6	18.7(0.0)	20.7(-4.6)	23.4(+0.9)
12	18.8(+0.5)	20.4(-6.0)	23.3(+0.4)
24	18.5(-1.1)	19.4(-10.6)*	23.5(+1.3)
48	18.8(+0.5)	18.7(-13.8)*	24.2(+4.3)

* $p<0.5$, Dunnett's t test

Postmortem Maternal and Fetal Examinations

Maternal examinations included gross macroscopic examination of all internal organs; each uterus was examined for content and fetal position; corpora lutea were counted. Each fetus was removed from the uterus, sexed, weighed and examined for gross external abnormalities.

One third of the live fetuses from each litter were fixed in a mixture of ethanol, formol and acetic acid for subsequent soft tissue examination (technique of Wilson).

The remaining two thirds of live fetuses were cleared in a solution of potassium hydroxide and stained with alizarin red for subsequent skeletal examination (modified technique of Dawson).

The uteri (and contents) of all pregnant females were weighed on the scheduled day of necropsy and used to determine the corrected body weight gain. The uteri of non-pregnant females were placed in an aqueous solution of ammonium sulfide to detect/accentuate possible resorption sites.

Results

Macroscopic Maternal Examination

No treatment-related change was noted in any female.

Uterine Examination (data on appended pages 12 & 13)

Differences between corpora lutea counts, which were comparable between all groups, and implantation site counts showed that the mean number of pre-implantation losses was increased slightly among females in the 48 mg/kg group, however, these losses occurred prior to the scheduled dosage period. Evidence of embryo-/fetal toxicity at the 24 and 48 mg/kg dosage levels was seen as follows (numerical differences relative to mean control values):

1/ Total number of fetuses/dam (alive + dead)decreased*†
2/ Number of live fetuses/dam decreased*
3/ Increased proportion and number* of resorptions
4/ Proportion of post implantation losses increased†

*$p<0.05$, ANOVA based on Wilcoxon ranks

External Fetal Examinations

The following anomalies were observed during this examination:

1/ Runts- 1 fetus in each of the 6, 12, 24 and 48 mg/kg dosages groups

2/ Hydrocephaly†- 1 fetus in the 48 mg/kg group

3/ Cleft palate†- 2 fetuses (2 litters) in the 48 mg/kg group

† Different from historical control incidence(Appended pages 14, 15, 16 & 17)

Sex Ratios

Sex ratios were comparable between all groups.

Fetal Body Weights

Mean fetal body weights were decreased in the 24 and 48 mg/kg groups. Although these weights were only 8.3% below the control value, the differences were statistically significant.

Dosage group mg/kg	0	6	12	24	48
Mean B.W. (g)	4.8	4.8	4.7	4.4*	4.4*
S.D.	0.4	0.4	0.5	0.7	0.7

* $p<0.05$, Analysis of Variance based on Wilcoxon ranks

Soft Tissue Examinations

The following anomalies were observed during this examination:

1/ Hydrocephalus internus†- 1 fetus in the 24 mg/kg and 2 fetuses (2 litters) in the 48 mg/kg groups(both cerebral hemispheres involved in each fetus); hydrocephaly was previously observed in one of these highdose fetuses during the external examination.

2/ Cleft palate†- this anomaly was observed in 2 high-dose fetuses (2 litters) during the external examination and confirmed by this examination.

Skeletal Examinations

Malformations

No treatment-related malformations were observed.

Variations

The only skeletal variations which appeared to be treatment-related were the presence of supernumerary ribs (no. 14 both sides), especially in the 12, 24 and 48 mg/kg groups, and an increased incidence of incompletely ossified phalangeal nuclei and calcanea with still absent ossification, most prominent in the 24 and 48 mg/kg groups(appended pages 18 & 19).

Supernumerary ribs(no. 14)

Dosage group (mg/kg)	0	6	12	24	48
			No. of fetuses (%)		
Left side	3(1.8)	10(5.9)*	17(9.4)**	30(20.5)**	34(29.1)**
Right side	4(2.4)	9(5.3)	14(7.8)*	29(19.9)**	31(26.5)**

* $p<0.05$, ** $p<0.01$, Fisher's exact

† Different from historical control incidence(Appended pages 14, 15, 16 & 17)

Statistical Analyses

The statistical methods used in this study are described on appended page 20.

Discussion and Conclusions

A suspension of Cyproconazole in distilled water mixed with carboxymethylcellulose sodium salt (CMC, 4%) was administered daily to pregnant Wistar/Han rats (25/group) via oral gavage from day 6 through 15 of gestation at dosage levels of 6, 12, 24 and 48 mg/kg.

Evidence of maternal toxicity included inhibited body weight gain during treatment at dosage levels of 12 mg/kg and above and decreased body weight and food consumption among females in the 24 and 48 mg/kg dosage groups. However, since corrected body weight gains were comparable between all groups, the evidence for maternal toxicity is equivocal.

Evidence of fetal toxicity was apparent from observed dose-related increases in the number of fetuses with supernumerary ribs at dosages of 12 mg/kg and above. Embryo/fetal toxicity was apparent at 24 and 48 mg/kg from the following observations: decreased total number of fetuses/dam, increased number of dead fetuses/dam, decreased number of live fetuses/dam, decreased percentage of implantations alive, increased percentage of fetal resorptions, increased percentage of post implantation losses, decreased body weight and incomplete ossification in phalangeal nuclei and the absence of ossification in calcanea. An increase in pre-implanation losses was observed at the 48 mg/kg dose only, prior to the scheduled dosing period. It was indicated in this evaluation that some of these changes were different from the historical control values provided by the test facility, however, the historical data were not presented in a format that would make a comparison of all of the investigated parameters possible. Also, the time frame in which the historical data were generated was not indicated. There was evidence to indicate that embryo/fetal toxicity was induced at the 6 mg/kg level. Although one runt was observed at this dose, the occurrence of 1 runt in each of the other dosage groups with no dose-response relationship probably indicates that this observation was incidental. A slight increase in the number of fetuses with supernumerary ribs was observed at this dose level and the increase was statistically significant on one side.

There was evidence of teratogenicity in the 24 and 48 mg/kg groups. Hydrocephaly was observed in 1 fetus in the 24 mg/kg and 2 fetuses in the 48 mg/kg groups. This malformation was not observed in the pilot study, however, since soft tissue examinations were not performed in that study, hydrocephalus internus would not have been detected. Cleft palate was observed in 2 fetuses in the 48 mg/kg group only. Cleft palate was also seen in the pilot study at dosage levels of 30, 75 and 120 mg/kg.

Although the noted malformations occurred at dosages that induced possible maternal toxicity, the changes indicating toxicity (decreased body weight, body weight gain and food consumption) were not severe and evidence of maternal stress was not remarkable. Also, there was evidence that the noted differences in maternal body weights were influenced by intrauterine effects (e.g., increased resorptions, decreased fetal weights etc.). Therefore, the evidence does not support the conclusion that these malformations are secondary effects from induced maternal toxicity.

The NOEL for developmental toxicity was not clearly apparent in this study since an increased fetal incidence of supernumerary ribs was observed at 6 mg/kg. The litter incidence of this effect could not be determined from the submitted data, therefore, the registrant should submit data showing the litter incidence of supernumerary ribs (number of litters/group with this change) with appropriate statistical analyses to aid in the determination of a possible NOEL for developmental toxicity in this study.

Developmental toxicity NOEL: not attained
Maternal toxicity NOEL: 6 mg/kg (equivocal)

007003

Core classification - supplemental (can possibly be upgraded to minimum by submitting requested data)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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FEB - 5 1990

OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: CYPROCONAZOLE-SUPPLEMENTAL DATA FOR A DEVELOPMENTAL TOXICITY STUDY IN RATS.

TO: S. LEWIS/GRABLE PM 21
REGISTRATION DIVISION (H7505C)

FROM: K. CLARK SWENTZEL K. Clark Swentzel 2/2/90
SECTION HEAD
TOXICOLOGY BRANCH II (HFAS)
HED (H7509C)

THRU: MARCIA VAN GEMERT, PH.D. Marcia van Gemert 2/5/90
CHIEF
TOXICOLOGY BRANCH II (HFAS)
HED (H7509C)

EPA ID No. 55947-RGG
MRID No. NONE
PROJECT No. 9-2120
CASWELL No. 272E
REGISTRANT: SANDOZ CORP.

REQUESTED ACTION

REVIEW SUPPLEMENTAL DATA.

TB II REVIEW OF SUBJECT STUDY

THE PRESENT SUBMISSION IS THE REGISTRANT'S RESPONSE TO THE TB II REVIEW (MEMORANDUM, SWENTZEL, HED, TO ROSSI, RD, JANUARY 17, 1989) OF A DEVELOPMENTAL TOXICITY STUDY (STUDY No. 048712) IN WHICH A SUSPENSION OF CYPROCONAZOLE TECHNICAL IN DISTILLED WATER MIXED WITH CARBOXYMETHYLCELLULOSE SODIUM SALT (CMC, 4%) WAS ADMINISTERED DAILY TO PREGNANT WISTAR/HAN RATS (25/GROUP) VIA ORAL GAVAGE FROM DAY 6 THROUGH 15 OF GESTATION AT DOSAGE LEVELS OF 6, 12, 24 OR 48 MG/KG.

IT WAS CONCLUDED IN THE REVIEW THAT ALTHOUGH INHIBITED BODY WEIGHT GAIN DURING TREATMENT AMONG FEMALES IN THE 12, 24 AND 48 MG/KG/DAY GROUPS WAS EVIDENCE OF MATERNAL TOXICITY, THE NOTED BODY WEIGHT DIFFERENCES APPEARED TO BE INFLUENCED BY TREATMENT-RELATED INTRA-UTERINE EFFECTS (INCREASED NUMBER OF RESORPTIONS AND DECREASED FETAL WEIGHT). THEREFORE, TB II CONSIDERED 6 MG/KG/DAY TO BE AN EQUIVOCAL NOEL FOR MATERNAL TOXICITY. ALSO, A NOEL FOR DEVELOPMENTAL TOXICITY WAS NOT ESTABLISHED BECAUSE THERE WAS AN INCREASED FETAL INCIDENCE OF SUPERNUMERARY RIBS OBSERVED AT 6 MG/KG/DAY, THE LOWEST DOSE ADMINISTERED, SO THE STUDY WAS CLASSIFIED CORE-SUPPLEMENTARY.

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HOWEVER, IT WAS INDICATED THAT THE STUDY MIGHT BE UPGRADED IF THE REGISTRANT COULD SUBMIT DATA SHOWING THE LITTER INCIDENCE OF SUPERNUMERARY RIBS WITH APPROPRIATE STATISTICAL ANALYSES TO AID IN THE DETERMINATION OF A POSSIBLE NOEL FOR DEVELOPMENTAL TOXICITY.

REGISTRANT'S RESPONSE

MATERNAL TOXICITY

THE REGISTRANT INDICATED THAT IT WAS "INAPPROPRIATE" TO RELY ON FINAL CORRECTED BODY WEIGHT GAIN (I.E., TOTAL WEIGHT GAIN DURING TREATMENT LESS TOTAL INTRA-UTERINE WEIGHT) TO DETERMINE MATERNAL TOXIC DOSAGE LEVELS. THIS POSITION WAS BASED ON INHIBITED BODY WEIGHT GAIN (-18, -29 AND -35% OF CONTROL AT 12, 24 AND 48 MG/KG, RESPECTIVELY, DURING THE INITIAL DAYS OF TREATMENT (GESTATION DAYS 6-11) AND THE ABSENCE OF INTRA-UTERINE EFFECTS AT 12 MG/KG/DAY. ALTHOUGH IT CAN BE ARGUED THAT THE THE INHIBITED BODY WEIGHT GAIN OBSERVED AMONG FEMALES IN THE 24 AND 48 MG/KG GROUPS MAY BE ATTRIBUTED TO PREVIOUSLY NOTED INTRA-UTERINE EFFECTS, IT IS TB II'S OPINION THAT, WITH CONSIDERATION OF THE REGISTRANT'S ARGUMENTS, 6 MG/KG CAN BE ESTABLISHED AS A NOEL FOR MATERNAL TOXICITY. HOWEVER, TB II STILL CONSIDERS THE MATERNAL TOXIC EFFECTS OBSERVED IN THIS STUDY (PREVIOUSLY NOTED INHIBITED BODY WEIGHT GAIN DURING TREATMENT AND DECREASED FOOD CONSUMPTION AT THE 24 AND 48 MG/KG DOSAGES) TO BE MINIMAL TOXIC EFFECTS, SO THE DEVELOPMENTAL EFFECTS OBSERVED IN THIS STUDY (SEE BELOW) SHOULD NOT BE CONSIDERED SECONDARY EFFECTS FROM MATERNAL TOXICITY.

SUPERNUMERARY RIBS

THE REGISTRANT SUBMITTED THE FOLLOWING LITTER INCIDENCE DATA FOR SUPERNUMERARY RIBS:

DOSAGE(MG/KG/DAY)	0	6	12	24	48
INCIDENCE	6/23	6/22	9/23	16/25†	16/21*
%	26	27	39	64	76

* $p < 0.05$, FISHER'S EXACT
† REIGISTRANT INDICATED 17/25(68%); THIS DOES NOT CORRELATE WITH TABULATED DATA

THESE DATA SHOW THAT THE LITTER INCIDENCE OF THIS VARIATION AT THE LOW-DOSE LEVEL IS COMPARABLE TO THAT FOR THE CONTROLS, THEREFORE, A NOEL FOR DEVELOPMENTAL TOXICITY CAN NOW BE ESTABLISHED. THE REGISTRANT CONCLUDED THAT THE NOEL FOR THIS EFFECT IS 24 MG/KG/DAY SINCE ONLY THE INCREASED INCIDENCE AT 48 MG/KG/DAY IS STATISTICALLY SIGNIFICANT. IT IS TB II'S OPINION THAT THE NOEL FOR THIS EFFECT IS 6 MG/KG/DAY. ALTHOUGH THE INCREASE AT 12 MG/KG/DAY IS NOT STATISTICALLY SIGNIFICANT, IT IS A SUBSTANTIAL INCREASE THAT IS DOSE-RELATED TO INCREASES OBSERVED AT THE 2 HIGHEST DOSAGES. THEREFORE, 12 MG/KG/DAY IS CONSIDERED THE LEL FOR DEVELOPMENTAL TOXICITY IN THIS STUDY.

HYDROCEPHALY AND CLEFT PALATE

HYDROCEPHALUS INTERNUS WAS SEEN IN 1 FETUS AT 24 MG/KG AND 1 FETUS AT 48 MG/KG WHILE HYDROCEPHALUS EXTERNUS OCCURRED IN 1 FETUS AT 48 MG/KG; BOTH CEREBRAL HEMISPHERES

WERE INVOLVED IN EACH FETUS AND THE EFFECTS IN THE HIGH-DOSE GROUP WERE SEEN IN 2 LITTERS. THE REGISTRANT INDICATED THAT THE NOTED HYDROCEPHALUS INTERNUS MIGHT ACTUALLY HAVE BEEN DELAYED DEVELOPMENT OF THE LATERAL VENTRICLES OF THE BRAIN WHICH WOULD BE REVERSIBLE WITH CONTINUED GROWTH. IT IS TB II'S OPINION THAT THERE IS NO MEANS TO VERIFY THIS SPECULATION FROM THE AVAILABLE INFORMATION.

CLEFT PALATE WAS OBSERVED IN 2 FETUSES (2 LITTERS) IN THE 48 MG/KG GROUP. THE REGISTRANT INDICATED THAT THE INCIDENCE WAS NOT STATISTICALLY SIGNIFICANT WHEN COMPARED WITH CONTROL VALUES AND THAT THIS MALFORMATION (AS WELL AS HYDROCEPHALY) OCCURRED ONLY AT DOAGES THAT WERE TOXIC TO THE FEMALE. TB II BELIEVES THAT IT SHOULD BE NOTED THAT THE OBSERVED INCIDENCES OF HYDROCEPHALY AND CLEFT PALATE BOTH EXCEEDED RESPECTIVE VALUES REPORTED IN THE REGISTRANT'S HISTORICAL CONTROL DATA (APPENDED PAGES 14-17, MEMORANDUM, SWENTZEL, HED, TO ROSSI, RD, JANUARY 17, 1989) AND THAT CLEFT PALATE WAS ALSO SEEN IN THE PILOT STUDY AT DOSAGES OF 30, 75 AND 120 MG/KG/DAY.

CONCLUSIONS

BASED ON ADDITIONAL INFORMATION IN THE CURRENT SUBMISSION, A NOEL FOR DEVELOPMENTAL TOXICITY CAN BE ESTABLISHED AT 6 MG/KG/DAY; THE LEL IS 12 MG/KG/DAY, BASED ON AN INCREASED INCIDENCE OF SUPERNUMERARY RIBS. HYDROCEPHALY, SEEN IN 1 FETUS AT 24 MG/KG AND 2 FETUSES AT 48 MG/KG, AND CLEFT PALATE, WHICH OCCURRED IN 2 FETUSES (2 LITTERS) AT 48 MG/KG, ARE CONSIDERED TO BE COMPOUND-INDUCED. ADDITIONAL EFFECTS INDICATIVE OF DEVELOPMENTAL TOXICITY AT 24 AND 48 MG/KG INCLUDED DECREASED LITTER SIZE. DECREASED NUMBER OF LIVE FETUSES/LITTER, DECREASED FETAL BODY WEIGHT, INCREASED RESORPTION RATE, INCOMPLETE OSSIFICATION IN PHALANGEAL NUCLEI AND THE ABSENCE OF OSSIFICATION IN CALCANEA. THE NOEL FOR MATERNAL TOXICITY IS ALSO 6 MG/KG/DAY, BASED ON INHIBITED BODY WEIGHT GAIN DURING TREATMENT AT 12 MG/KG. THE MATERNAL TOXICITY INDUCED IN THIS STUDY IS CONSIDERED MINIMAL, THEREFORE, TB II DOES NOT CONSIDER THE NOTED VARIATIONS AND MALFORMATIONS TO BE SECONDARY EFFECTS FROM MATERNAL TOXICITY.

TB II WILL RECOMMEND THAT THE DATA IN THIS STUDY SHOULD BE FURTHER EVALUATED BY THE HED DEVELOPMENTAL TOXICITY PEER REVIEW COMMITTEE.

CORE-CLASSIFICATION: THIS STUDY CAN BE UPGRADED TO CORE-MINIMUM

GUIDELINE REQUIREMENT: THIS STUDY SATISFIES THE DATA REQUIREMENT FOR GUIDELINE NO. 83-3(,

Reviewed by: K. Clark Swentzel K. Clark Swentzel, 11/18/88 007003
Section 2 , Tox. Branch (TS-769C)
Secondary reviewer: James N. Rowe, Ph.D. James N. Rowe 11/23/88
Section 2 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Teratogenicity Study in Rabbits TOX. CHEM. NO.: 272E

MRID NO.: 406077-20

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol
SYNONYMS: Cyproconazole; SAN 619F

STUDY NUMBER(S): 053886

SPONSOR: Sandoz Corp.

TESTING FACILITY: Research and Consulting Company, A.G., Itingen, Switzerland

TITLE OF REPORT: Teratogenicity Study in Rabbits with SAN 619F

AUTHOR(S): H. Becker

REPORT ISSUED: March 21, 1986

TEST DATES: October 21, 1985 - December 4, 1985

CONCLUSIONS

A suspension of Cyproconazole in distilled water mixed with carboxymethylcellulose sodium salt (CMC, 4%) was administered daily to pregnant Chinchilla rabbits (16/group) via oral gavage from day 6 through 18 of gestation at dosage levels of 2, 10 and 50 mg/kg.

Evidence of maternal toxicity, which was not remarkable, included inhibited body weight gain during treatment and decreased food consumption during the initial phase of treatment, both at 50 mg/kg. However, since corrected body weight changes between groups were comparable, the evidence of compound-induced maternal toxicity in this study is not convincing.

Embryo/fetal toxicity, observed at 50 mg/kg, was evident from the decreased number of live fetuses/dam and an increased incidence of non-ossification in certain forelimb and hind limb digits. Evidence of embryo/fetal toxicity at dosages of 10 and 50 mg/kg was indicated by an increased incidence of embryonic and fetal resorptions.

Evidence of teratogenicity included hydrocephalus internus, observed in 1 fetus at each dosage level, and agenesia of the left kidney and ureter in 1 high-dose fetus. Hydrocephaly was also seen at 2 dosage levels in a developmental toxicity study in rats with this test material, however, this anomaly did not occur in the control group of either study.

Since a teratogenic response to the test material was observed at the lowest dose tested, a NOEL for developmental toxicity was not attained in this study. Although evidence of maternal toxicity at 50 mg/kg was not remarkable, the 10 mg/kg dosage level is clearly a no-effect level for maternal toxicity.

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This study is not acceptable for regulatory purposes because: 1) a NOEL for developmental toxicity apparently was not attained and 2) the concentrations of test material were not within the acceptable ± 15% of nominal concentration for the mid- and highdose suspensions immediately after preparation.

Developmental toxicity NOEL: not attained ; < 2 mg/kg/day (LDT)
Maternal toxicity NOEL: 10 mg/kg (equivocal)

Core classification: supplementary

Quality assurance statement: signed and dated by the QAU

RANGE-FINDING STUDY

The main teratogenicity study was preceded by a pilot study, which was performed to determine appropriate dosage levels of Cyproconazole for the main study. This study was also performed by RCC.

Study Title: Dose-Finding Teratogenicity Study in rabbits with SAN 619F

Study No.: 048701

Date of report: October 2, 1985

Date of study: August 12, 1985 - September 10, 1985

Author: H. Becker

Materials and Methods

Cyproconazole(Batch No. 8507, purity-95.6%) was administered to mated female hybrid Chinchilla rabbits (3/group) via gavage at dosage levels of 0(vehicle: distilled water with 4% carboxymethylcellulose sodium salt, 99.5%), 1, 4, 12 and 40 mg/kg on days 6 through 18 of gestation. The dams were sacrificed on day 28 of gestation; postmortem examinations included macroscopic inspection of internal organs and uterus (uterine contents and the position of each fetus) as well as corpora lutea counts. The uteri (and contents) of all pregnant females were weighed to determine corrected body weights. The fetuses were sexed, weighed, examined for external gross abnormalities and prepared for internal examinations which included body cavities (thorax, abdomen, pelvis) and the enclosed organs. The crania were examined for ossification.

Results

Maternal toxicity was evident at 40 mg/kg from decreased food consumption on days 6-11 and 24-28 and a slightly lower mean body weights (7 to 8% below controls) during the treatment period.

Evidence of fetal toxicity at 40 mg/kg included a decrease in the proportion of live fetuses and increases in the proportions of fetal resorptions and post implantation losses. Results of cranial and body cavity examinations were not tabulated, however, the investigator indicated that agenesia of the left or right kidney and ureter was observed in 2 fetuses from 1 litter from the 40 mg/kg group.

Conclusions

Since the noted effects reflecting maternal toxicity at 40 mg/kg were not remarkable, a higher dosage level was selected for the main study. The dosage levels of Cyproconazole chosen for the main teratogenicity study were 2, 10, and 50 mg/kg/day. Based on the treatment-related effects observed in this study, the selected dosages appear to be appropriate.

Core classification: Supplementary data (Range-finding study)

Main Developmental Toxicity Study

TEST MATERIAL

Concentration of technical material = 95.6%; Lot no.: 8507; Code name: SAN 619 F; Isomer composition: not reported; Description: brown-beige powder.

TEST ANIMAL DATA

Rabbits: Chincilla; 64 mated females, 16/group; Age at initiation of study = between 4 & 5 months; Body weights at study iniation (post coitum): 2574-3661g; Identification: individual cage numbers and corresponding number inscribed in ear.

Group assignment: 16 females were randomly assigned to 1 control and each of 3 dosage groups.

Acclimation: 7 days

Housing: Individually in stainless steel cages equiped with automatic cleaning system.

Food: Pelleted Kliba 341 rabbit maintenance diet, ad libitum.

Water: municipal tap water: ad libitum

Environmental parameters: Light - 12 hr light/dark cycle; Temperature - 22 ± 3°C; Relative humidity - 40 - 70%; 10-15 air changes/hr; monitored hourly.

METHODS

Mating

Following acclimation, the females were housed with males(1:1) until mating had been observed (designated day 0 post coitum). Each mated female was housed individually.

Preparation of Test Material

The test material/vehicle mixtures were prepared daily prior to administration. The vehicle, distilled water with carboxymethylcellulose sodium salt (CMC; 4%), was added to a weighed amount of Cyproconazole; homogeneity was maintained by constant stirring. The concentration of test compound was not indicated.

Administration of Test Material

The test material was administered daily via oral gavage from day 6 through day 18 of gestation at nominal dosage levels of 2, 10 and 50 mg/kg. The controls received vehicle only. All groups received a volume of 4 ml/kg body weight with a daily adjustment of individual volume to the actual body weight.

Test Mixture Analysis

Determination of concentration as well as the homogeneity and stability of the test mixtures were performed once during the treatment period. Samples were taken immediately after mixture preparation and again after 90 minutes.

Results

The measured concentrations of Cyproconazole ranged from 73.3 to 93.3% and 48.8 to 80.0% of nominal concentrations immediately after preparation and 90 minutes after preparation, respectively(data on appended page 1). The test material concentrations in the dosage suspensions for the 10 and 50 mg/kg groups were not within acceptable limits (± 15% of nominal concentration) immediately after preparation. Ninety minutes after preparation, none of the dosage suspension/test material concentrations were within acceptable limits. Triplicate analyses of each dosage suspension also showed that these mixtures were not homogeneous.

Maternal Mortality

Animals were observed twice daily for possible mortalities.

Results: None of the females died during the study.

Signs and Symptoms

Animals were examined twice daily.

Results: No apparent treatment-related clinical signs or symptoms were observed. One female in the 2 mg/kg group aborted on day 28 p.c. Three dead fetuses (not autolytic) and 3 fetal resorptions were found. The investigator indicated that no anomalies or malformations were found during the external examination of the 3 dead fetuses.

Number of Dams Examined

Only dams with live fetuses on day 21 post coitum were included in the calculations of body weight gain, corrected body weight gain, mean daily food consumption and reproduction data.

Dose (mg/kg)	0	2	10	50
No. of Dams	15	14	16	16

Maternal Body Weight and Body Weight Gain

Body weights were measured daily from day 0 until day 28 post coitum. Calculations of body weight gain during the treatment period started on day 6 post coitum (immediately prior to the first administration) and ended on day 19 post coitum (approximately 24 hr after the last administration).

Results: A comparison of mean body weights between groups did not reveal an obvious treatment-related effect. Initially, the mean weight of the high-dose group was approximately 6% lower than the control value; this deficit was consistent thoughout most of the study but increased slightly to -10.7% on day 28 p.c.

Data for mean group body weight gains (appended page 2) show that the mean weight gain in the high-dose group exceeded that of the controls during the periods before and after treatment (days 0-6 and 19-28) but was lower (-37.6%) during treatment, implicating a possible compound-related effect. Body weight gains in the other treatment groups either exceeded or were comparable to control values at most intervals.

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Corrected Body Weight Gain

Corrected body weight gain was calculated as follows:

[Body weight on gestation day 28 - Body weight on gestation day 6 - Uterus weight at necropsy on gestation day 28]

Results: Corrected body weight gains were comparable between treated and control groups. Therefore, the noted decreases in body weight and body weight gain among dams in the treatment groups were apparently influenced by increased resorptions (see fetal data below).

Mean corrected body weight gain

Dose (mg/kg)	0	2	10	50
% change rel. to day 6 b.w.	-4.9	-4.5	-3.7	-4.9

Food Consumption

Food consumption was determined on days 6, 11, 15, 19, 24 and 28 of gestation.

Results: Food consumption was decreased (-26.9%, $p<0.05$, Dunnett-test) in the high-dose group during the initial phase of the treatment period (days 6 - 11). Noted differences between treated and control groups at other intervals were not remarkable (appended page 3).

Postmortem Maternal and Fetal Examinations

Maternal examinations included gross macroscopic examination of all internal organs; each uterus was examined for content and fetal position; corpora lutea were counted. Each fetus was removed from the uterus, sexed, weighed and examined for gross external abnormalities and prepared for internal examinations.

The following examinations were performed on each fetus:

1) All fetuses were dissected for body cavity (thorax, abdomen and pelvis) examination; the organs were examined for abnormalities.

2) The skin was removed from the crania of all fetuses in order to examine for ossification.

3) Following fixation in trichloroacetic acid and formaldehyde, the heads were cross-sectioned and the cephalic viscera were examined.

4) The trunk of each fetus was placed in a solution of potassium hydroxide for clearing and stained with alizarin red for subsequent skeletal examination.

Results

Macroscopic Maternal Examination

No treatment-related change was noted in any female.

Uterine Examination (data on Appended pages 4 & 5)

Embryo/fetal toxicity was evident from the following observations:

1/ Total number of fetuses/dam (alive + dead)decreased† - high-dose
2/ Number of live fetuses/dam decreased† - high-dose
3/ Increased proportion and number of resorptions - mid-dose† and high-dose*†

*p<0.05, ANOVA based on Wilcoxon ranks
† Different from historical control incidence(Appended page 6)

External Fetal Examinations

The noted anomalies (thoracogastroschisis, omphalocele and shortened tail) were isolated and had no apparent relationship to treatment.

Sex Ratios

Sex ratios were unrelated to dosage levels of test material.

Fetal Body Weights

Group mean fetal weights were comparable between all groups.

Body Cavity Examinations

The following anomalies were observed during this examination:

1/ Agenesia of the diaphragm- 1 fetus in the control group†† and 1 fetus in the 10 mg/kg group††.

2/ Agenesia of the left-kidney and ureter — 1 fetus in the 50 mg/kg group††.

These isolated findings were not clearly associated with treatment. Agenesia of the left kidney and ureter was noted for 8/3202 fetuses in the historical control data.

Cranial Examination

No abnormalities were noted for any fetus. It was indicated that the stage of ossification was similar between groups.

Cephalic Viscera Examination

Anomalies found:

1/ Microphthalmia- 4 fetuses in one litter in the 10 mg/kg group††.

2/ Hydrocephalus internus- 1 fetus in each dosage group†† (both hemispheres)

††Different from historical controls (Appended pages 7,8,9,10,11 & 12)

Skeletal Examinations

Malformations

The observed malformations were isolated, not dosage-related and could not be conclusively associated with treatment(appended page 13).

Variations

An increased incidence of non-ossification of digit no. 5 of the forelimbs and digit no. 4 of the hind limbs was observed among fetuses in the high-dose group (Appended pages 14 & 15). Since this variation was not seen in the controls or the lower dosage groups, it may be associated with treatment.

Statistical Analyses

The statistical methods used in this study are described on appended page 16.

Discussion and Conclusions

Cyproconazole, suspended in distilled water mixed with carboxymethylcellulose sodium salt (CMC, 4%), was administered daily to pregnant Chinchilla rabbits (16/group) via oral gavage from day 6 throug 18 of gestation at dosage levels of 2, 10 and 50 mg/kg.

Evidence of maternal toxicity, which was not remarkable, included inhibited body weight gain during treatment and decreased food consumption during the initial phase of treatment, both at 50 mg/kg. However, since corrected body weight changes between groups were comparable, the evidence of compound-induced maternal toxicity in this study is not convincing.

Embryo/fetal toxicity, observed at 50 mg/kg, was evident from the decreased number of live fetuses/dam and an increased incidence of non-ossification in certain forelimb and hind limb digits. Evidence of embryo/fetal toxicity at dosages of 10 and 50 mg/kg was indicated by an increased incidence of embryonic and fetal resorptions.

There was evidence of teratogenicity at all dosage levels. Although hydrocephalus internus was seen in only 1 fetus at each dosage level, this anomaly was also observed at 2 dosage levels in a developmental toxicity in rats (Teratogenicity Study in Rats with SAN 619F; MRID No. 406077-21). Furthermore, hydrocephaly was not seen in the control group of either study. The spontaneous incidence of this anomaly among this strain of rat at the test facility was only 9/10,935 (0.08%). Agenesia of the left kidney and ureter was observed in 1 fetus at 50 mg/kg, however, kidney and ureter agenesia was also observed in 2 fetuses (1 litter) at 40 mg/kg in the pilot study. Therefore, this malformation may be treatment-related as well.

Since a possible teratogenic response to the test material was observed at the lowest dose tested, a NOEL for developmental toxicity was not attained in this study. Although evidence of maternal toxicity at 50 mg/kg was not remarkable, the 10 mg/kg dosage level is clearly a no-effect level for maternal toxicity.

This study is not acceptable for regulatory purposes because: 1) a NOEL for developmental toxicity apparently was not attained and 2) the concentrations of test material were not within the acceptable ± 15% of nominal concentration for the mid- and highdose suspensions immediately after preparation.

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007003

Developmental toxicity NOEL: not attained; <2 mg/kg/day (LDT)
Maternal toxicity NOEL: 10 mg/kg (equivocal)

Core classification: supplementary

Reviewed by: K. Clark Swentzel K. Clark Swentzel 12/2/88 007003
Section 2 , Tox. Branch (TS-769C)
Secondary reviewer: James N. Rowe, Ph.D. James N. Rowe 12/7/88
Section 2 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Two-Generation Reproduction in Rats TOX. CHEM. NO.: 272E

MRID NO.: 406077-23

TEST MATERIAL: alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol
SYNONYMS: Cyproconazole; SAN 619F

STUDY NUMBER(S): 6712/87

SPONSOR: Sandoz Corp.

TESTING FACILITY: Sandoz Ltd., Agrotoxicology, Basel, Switzerland

TITLE OF REPORT: San 619F, 2-Generation Study in Rats

AUTHOR(S): B. Eschbach, R. Aerni, J.C. Karapally, R. Bourry

REPORT ISSUED: July 8, 1987

TEST DATES: February 27, 1986 - December 1986

CONCLUSIONS

Four groups of KFM-Wistar rats were administered technical Cyproconazole at dietary levels of 0(control), 4, 20 and 120 ppm during the pre-mating (10 weeks and 12 weeks, respectively, for the F_0 and F_1 generations), mating, pregnancy and lactation periods to assess the potential reproductive toxicity of the test compound.

Two of the reproductive parameters investigated in parental animals were affected by treatment in F_0 rats only: the duration of gestation at the mid- and high doses was increased and a lower number of implantation sites was seen in high-dose females, both in comparison to respective concurrent control values. Evidence of liver toxicity was seen in high-dose F_0 males (increased lipid storage and relative weight) and females (increased relative weight).

Parameters examined among the offspring which showed treatment-related effects included decreased litter sizes in both the F_1 and F_2 high-dose groups and the F_1 mid-dose group during the early phase of lactation (litters were standardized at day 4 post partum), decreased live birth index in the high-dose F_1 offspring and decreased viability index in the high-dose F_1 and F_2 offspring.

Based on the increased duration of gestation in F_0 dams and the decreased litter sizes observed in F_1 offspring, the LEL in this study was 20 ppm and the NOEL was 4 ppm, which correspond to approximate average dosage levels of 1.7 and 0.4 mg/kg/day, respectively.

Core-classification: minimum (provided test compound stability data and and a description of the sampling technique used for the analyses of dietary levels of test compound are submitted)

Quality assurance statement: signed and dated by the QAU

Test Material

Technical purity: 95.6%; Lot No.: 8507; stereoisomer composition: A=49%, B=51%; description: brownish powder.

Diet

Commercial powdered rat diet (KLIBA No. 21-343-4), provided ad libitum.

Drinking Water

Municipal water in polypropylene bottles, provided ad libitum.

Test Animals

KFM-Wistar albino rats; age at the initiation of treatment: 8 weeks; 26/sex/group.

Acclimation Period

Two weeks

Identification (parent generations)

Cage cards and individual ear marking

Housing

Individually (except during mating period) in Macrolon® polycarbonate cages (size 3), equipped with food and water dispensers; bedding was heat sterilized fine hard wood chips.

Environmental Parameters

Temperature: 23 ± 2°C; relative humidity: 50 ± 20%; light cycle: 12/12; air changes: 15/hr.

METHODS

Group assignment

F_0-generation
Parental animals were randomly allocated to 4 groups.

F_1-generation
Twenty six male and 26 female pups were randomly drawn from each group (1 male and 1 female/litter when possible) for the subsequent part of the study. Brother/sister pairing was avoided.

Dosage groups
Dietary levels of Cyproconazole were 0(control), 4, 20 and 120 ppm.

Diet preparation
Test diet mixtures were prepared weekly from a 1% premix which was prepared at monthly intervals.

Stability of test material in the diet
The investigator indicated that data from a separate study showed that the test material was stable in the diet mixture for 14 days. However, these data, which were not included in the report, should be submitted.

Quantitation of test material in the diet
Samples were analysed at the start of the study and at 6 month intervals thereafter. The sampling technique used in these analyses was not described.

Results
Considerable variation was seen in the 4 ppm mixtures (2 analyses showed concentrations at 27 and 45% above nominal), however, since all other measurements approximated the accepted ± 15% of nominal, the increased concentrations at 2 intervals for the low-dose mixture did not compromise the validity of the study (Appended page 1).

Treatment

F_0-generation
These animals were treated continuously for at least 70 days (10 weeks) prior to mating and throughout the mating period. Males were treated for approximately 3 weeks after termination of the mating period and females were treated throughout the gestation, parturition and lactation periods.

F_1-generation
Selected animals were treated from weaning (21st day post partum) for approximately 84 days prior to mating. Treatment continued until necropsy for all animals.

OBSERVATIONS

Parental Animals

Clinical signs
F_0 and F_1: All animals were examined daily for clinical signs of toxicity.

Results
F_0: The investigator indicated that one pregnant high-dose female, which failed to deliver, showed signs of distress such as increased respiration rate and piloerection. No other animal exhibited clinical signs.

F_1: No signs of toxicity were seen in any animal.

Mortalities
F_0 and F_1: A gross necropsy was performed on any animal killed in extremis during the study. Organs/tissues with gross abnormalities were fixed in 4% formalin for possible histologic examination.

Results
F_0: No parental animal died during the study. One mid-dose and one high-dose female were killed because of complete postnatal loss on days 1 and 5, respectively. Also, one pregnant high-dose female that failed to deliver was sacrificed on day 25 post coitum (p.c.).

F_1: No parental animal died during the study. One high-dose female was sacrificed because of complete postnatal litter loss on day 4 post partum (p.p.).

Body weights

F_0: Individual male body weights were recorded at weekly intervals throughout the study. Individual female body weights were recorded weekly during the pre-mating period, on days 0, 7, 14 and 20 p.c. and on days 0, 7, 14 and 21 p.p.

F_1: The selected F_1-animals were individually weighed at weekly intervals until mating. Pregnant and lactating females were recorded on the days indicated for corresponding F_0-females.

Results

F_0 and F_1: No treatment-related differences were evident between males or females and the respective controls during any stage of the study.

Food/compound consumption

F_0 and F_1: Individual food consumption was monitored at the same time as the body weight except during the mating period, when males and females had access to the same feeder.

Results

F_0 and F_1: No treatment-related differences were evident.

Based on food consumption data, the mean compound intake for the respective premating treatment periods are tabulated below:

Cyproconazole Ingestion Levels (mg/kg/day)

Dosage (ppm)	4		20		120	
Generation	Males	Females	Males	Females	Males	Females
F_0	0.28	0.33	1.39	1.67	8.29	9.88
F_1	0.37	0.45	1.77	2.16	10.88	13.30

Mating

F_0: After 10 weeks of dosing each male was paired with a female from the same dose group for a maximum of 21 days (3 weeks at 4 days/week). Vaginal smears were taken daily until sperm was found in the smear. The presence of sperm was recorded and the day of a sperm positive vaginal smear was considered day 0 of pregnancy. The female was removed on day 0 and caged individually.

Females which failed to mate were killed 26 to 28 days after the end of the mating period and subjected to gross and histologic examinations.

F_1: At approximately 15 weeks of age, each female was paired with a male from the same dose group but from a different litter. Vaginal smears were taken as indicated for the F_0-generation.

Results

F_0 and F_1: No treatment-related differences in precoital interval, copulation rate or pregnancy rate were observed in any group.

Parturition

F_0 and F_1: Females were observed at least 3 times/day from day 20 of gestation for signs of parturition. When parturition was observed, the times of onset and completion were recorded and post partum behavior of the female was observed. The day of completion of littering was called day 0 post partum.

Females which failed to deliver by day 25 p.c. or dams whose entire litters were born dead or which died prior to weaning were killed and subjected to necropsy.

Results

F_0: One high-dose pregnant female failed to deliver by day 25 p.c.

The following data indicate that parturition tended to occur later in F_0 treated groups than in corresponding controls (Appended page 2).

Pregnancy Length in F_0 Females

Dose group (ppm)	0	4	20	120
Delivery after day 22 p.c. (%)	7/22 (31.8)	9/23 (39.1)	12/22 (54.5)	11/22 (50.0)
Pregnancy length (days) Mean ± S.D.	22.3 0.5	22.4 0.5	22.6 0.6	22.5 0.5

Although the mean pregnancy length in each treated group exceeded the control value, only the durations shown for the mid- and high-dose groups exceeded the upper-range value from the investigator's historical control data (Appended pages 9 and 10). Therefore, the increased duration of gestation in these groups may be treatment-related.

F_1: Treatment had no apparent effect on parturition or the duration of gestation in F_1 dams.

Necroosy and Histopathology

F_0: The males were killed at approximately 3 weeks after the end of the mating period and the females at the time of the weaning of the F_1 offspring (day 21 p.p.), unless otherwise indicated. These animals were necropsied and each uterus, which was examined for abnormalities and the number of implantation sites, as well as the vagina , cervix, uterus, ovaries, testes, epididymides, seminal vesicles, prostate, coagulating gland, pituitary gland, liver and any tissue/organ with an abnormal lesion were fixed in 4% formalin for possible histologic examination.

The liver of each animal killed at scheduled termination was weighed.

Histologic examinations of noted tissues were performed on all control and high-dose animals as well as on any animal that failed to mate or deliver. Additionally, the liver of all F_0 low- and mid-dose males were examined because of findings in the high-dose group (see below).

F_1: The males were killed approximately 3 weeks after the end of the mating period and the females at the time of weaning of the F_2 offspring (day 21 p.p.). The necropsy was conducted as described for the F_0 animals.

Results

Histopathology

F_0: There was a statistically increase in the incidence of fatty change in the liver of high-dose males, characterized by the investigator as mainly macrovesicular lipid storage in hepatocytes of zones 3 and 2. The severity of this change appeared to be treatment, but not dosage, related.

Fatty Change in the Liver of F_0 Males

Dosage group (ppm)	0	4	20	120
Proportion (%)	10/26 (38.5)	12/26 (46.2)	12/26 (46.2)	19/26* (73.1)
Mean degree of severity	1.6	1.8	1.9	1.8

* $p < 0.05$, Fisher's Exact, Toxicology Branch

Liver weights

Mean absolute and relative liver weights in treated males and females were increased (Appended pages 3, 4, 5 and 6), however, only the increased relative weight in high-dose males was statistically significant ($p<0.05$, Kruskal-Wallis). However, the noted increases in relative weights may be treatment-related at the high-dose in both males and females.

Implantation sites

The mean number of implantation sites was decreased in the high-dose group(7.2% < controls), however, the value was within the historical control range.

Implantation Sites in F_0 Females

Dose group (ppm)	0	4	20	120
Mean	12.5	12.4	12.4	11.6
S.D.	1.6	3.1	1.6	2.5

Historical control range = 9.8-13.0

F_1: There was also a slight (not statistically significant) increase in the incidence of lipid storage in the liver of high-dose males (58% vs. 42% in controls).

All other macroscopic and microscopic changes observed in F_0 and F_1 parental animals appeared to be incidental.

OBSERVATIONS AND PROCEDURES (F_1 and F_2 Generations During Lactation)

Malformations

Gross malformations were noted and recorded. Grossly malformed pups were necropsied.

Results

No treatment-related malformations were evident.

Culling

On day 4 p.p. the size of each litter was adjusted by eliminating extra pups by random selection to yield, if possible, 4 males and 4 females. Litters of less than 8 pups were not altered. Culled pups were subjected to gross necropsy.

Litter size and sex

The litter size and pup sexes were recorded as soon as possible after birth and checked twice at day 0 p.p. and daily thereafter until day 21 p.p.

Results

Litter size

Mean litter sizes were smaller in the high-dose groups at days 0 and 4 p.p. in F_1 pups and at day 4 p.p. in F_2 pups. The mean litter size of the F_1 mid-dose pups was also lower than that of corresponding controls at day 4 p.p. The litter sizes at day 0 p.p. in all groups were within the historical control range; historical control data for litter sizes at day 4 p.p. were not provided in the report. The viability index was decreased in F_1 and F_2 pups and the live birth index was decreased in F_1 pups, all in high-dosage groups.

Litter Sizes at Days 0 and 4 (F_1 and F_2 pups/dam)

Dose group(ppm)		0	4	20	120
Day p.p.	Pups	Mean ; S.D.			
0	F_1	11.2 2.0	11.5 3.2	10.8 3.1	9.8 2.6
	F_2	11.0 2.8	11.7 2.9	12.0 2.2	10.9 2.7
[Historical control = 8.9 - 12.9]					
4	F_1	11.2 2.0	11.3 3.0	10.6 3.2	8.8 3.7
	F_2	10.7 2.7	11.2 2.8	11.8 2.2	10.2 3.5

Live Birth, Viability and Lactation Indices (%)

Dose group(ppm)		0	4	20	120
Index	Pups				
Live birth: # alive day 0 x 100 / total # day 0	F_1	97.3 (292/300)	98.9 (264/267)	99.6 (259/260)	95.9 (235/245)
	F_2	94.2 (242/257)	98.8 (257/260)	97.6 (287/294)	98.4 (239/243)
Viability: # alive day 4 x 100 / # alive day 0	F_1	99.7 (291/292)	98.1 (259/264)	98.1 (254/259)	93.2[1/] (219/235)
	F_2	97.5 (236/242)	94.2 (242/257)	98.9 (284/287)	93.3[2/] (223/239)
Lactation: # alive day 21 x 100 / # alive day 4	F_1	100.0 (244/244)	100.0 (176/176)	98.9 (178/180)	99.4 (171/172)
(standardized)	F_2	100.0 (170/170)	98.2 (167/170)	98.4 (185/188)	98.1 (156/159)

1/ One entire litter (11 pups) died.
2/ One entire litter (12 pups) died.

Sex
Treatment did not have an apparent effect on sex distribution in either F_1 or F_2 pups.

Clinical Signs
All pups were examined daily during lactation for clinical signs of toxicity. A necropsy was performed, where possible, on any pups dying or killed in extremis during lactation.

Results
No treatment-related clinical signs were noted.

Noted gross morphologic changes appeared to be incidental: 1 F_1 control pup did not have a tail, irregular skin development (partly hairless at days 9-14 p.p.) was seen in 1 F_1 mid-dose litter and 3 F_2 mid-dose litters.

Body Weights
Pup body weights were recorded by sex soon after birth and at days 4 (before and after culling), 7, 14, and individually on day 21 p.p.

Results
No treatment-related effects were observed at any of the investigated post-natal intervals in either F_1 or F_2 pups.

Histopathology

The necropsy procedure for F_1 animals was previously described. F_2 offspring were subjected to the same procedure after weaning.

Results

F_1: There was no evidence that the sporadic lesions observed were associated with treatment.

F_2: Hydronephrosis was a common finding in all groups; the data did not indicate that this or any other finding was induced by treatment.

STATISTICAL ANALYSES

The statistical methods used in this study are described on Appended pages 7 and 8.

DISCUSSION and CONCLUSIONS

Four groups of KFM-Wistar rats were administered technical Cyproconazole at dietary levels of 0(control), 4, 20 and 120 ppm during the pre-mating (10 weeks and 12 weeks, respectively, for the F_0 and F_1 generations), mating, pregnancy and lactation periods to assess the potential reproductive toxicity of the test compound.

Two of the reproductive parameters investigated in parental animals were affected by treatment in F_0 rats only: the duration of gestation at the mid- and high doses was increased and a lower number of implantation sites was seen in high-dose females, both in comparison to respective concurrent control values. Evidence of liver toxicity was seen in high-dose F_0 males (increased lipid storage and relative weight) and females (increased relative weight).

Parameters examined among the offspring which showed treatment-related effects included decreased litter sizes in both the F_1 and F_2 high-dose groups and the F_1 mid-dose group during the early phase of lactation (litters were standardized at day 4 post partum), decreased live birth index in the high-dose F_1 offspring and decreased viability index in the high-dose F_1 and F_2 offspring.

Based on the increased duration of gestation in F_0 dams and the decreased litter sizes observed in F_1 offspring, the LEL in this study was 20 ppm and the NOEL was 4 ppm, which correspond to approximate average dosage levels of 1.7 and 0.4 mg/kg/day, respectively.

Core-classification: minimum (provided test compound stability data and and a description of the sampling technique used for the analyses of dietary levels of test compound are submitted)

CYPROCONAZOLE

Page_____ is not included in this copy.

Pages 165 through 167 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

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U.S. ENVIRONMENTAL PROTECTION AGENCY
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TOX ONELINERS

TOXCHEM NO. 272E- Cyproconazole FILE LAST PRINTED: 05/14/90

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Teratology Species: rat Res. and Consulting Co.; Switz 048712; 12/11/85	Cyproconazole tech 95.6% Lot# 85067	406077-21	Levels tested in Han Wistar strain by gavage on 6 - 15 day of gestation - 0, 6, 12, 24, & 48 mg/kg. Maternal NOEL = 6 mg/kg (LDT; equivocal) , Maternal LEL = 12 mg/kg (based on inhibited body weight gain); Maternal toxicity was considered equivocal due to treatment-related intrauterine effects (e.g increased number of resorptions). Develop. NOEL = 6 mg/kg; Develop. LEL = 12 mg/kg ıcidence of supernumerary ribs); variations - 24 & 48 mg/kg 'e fetuses/litter, decr. fetal weight, incr. resorption rate fication of phalangeal nuclei & no ossification in calcanea. - Hydrocephaly; 24 & 48 mg/kg; At 48 mg/kg - cleft palate. upgraded. Satisfies Guideline # 83-3		Supplementary 007003 Minimum 007730
Teratology Species: rabbit Res. and Consulting Co.; Switz 053886; 3/21/86	SAN 619F tech 95.6%		= 10 mg/kg , Maternal LEL = 50 mg/kg (inhibited body ıring treatment & decrease food consumption [equivocal] al NOEL < 2 mg/kg. Embryo/fetal toxicity at 10 mg/kg ıcreased inc. of embryonic & fet. resorptions. Teratotive): hydrocephalus internus at all dose levels (2, 10, - 50 mg/kg), agenesis of the left kidney & ureter (50 mg/kg).		Supplementary 007003
Reproduction-2 generation Species: rat Sandoz Ltd 6712; 7/8/87	Cyproconazole tech 95.6% Lot# 8507	406077-23 412945-00	Levels tested in KFM Wistar strain - 0, 4, 20, & 120 ppm (0.4, 1.7, & 10.6 mg/kg). NOEL = 0.4 mg/kg , LEL = 1.7 mg/kg. Parameters affected at 1.7 mg/kg (mid-dose): increase duration of gestation (Fo only) & decrease litter sizes (F1 & F2). Test compound stability data and sampling techniques for dietary analyses are satisfactory (5/3/90).		Minimum 007003 007908
Teratology Species: rat Res. and Consulting Co.; Switz 048701; 7/9/85	Cyproconazole tech 95.6% pure Batch# 8507		Range Finding Study - Levels tested to Wistar KFK-HAN strain by gavage on 6 - 15 day of gestation: 0, 7.5, 30, 75, and 120 mg/kg. Maternal NOEL < 7.5 mg/kg (decrease food consumption) at 30 mg/kg - inhibited body weight gain. Developmental NOEL = 7.5 mg/kg , Developmental LEL = 30 mg/kg (increased early resorption, post implantation, decrease fetal body weight, and increase cleft palate)		Supplementary 007003
Feeding-1 year Species: dog Sandoz Ltd 394-D; 4/18/88	SAN 619 F tech. 95 +-1% (Cyproconazole)	412129-01	Levels in diet: 0, 30, 100, 350 ppm (1.0, 3.2, 12.1 mg/kg (M), 12.6 (F)); No effects on survival, ophthalmoscopic or hematologic parameters; slight decr. in body wt. gain at high-dose; dose-related increase in platelets in both sexes throughout study (not always statistifically significant); liver is target organ; absolute & relative liver weights increased in both sexes, but stat. sign. only in males; stat. sign. increases in cytochrome P450 in both sexes at high dose & in mid-dose females. The NOEL can be set at 30 ppm (1.0 mg/kg, the LEL at 100 ppm (3.2 mg/kg), based on liver effects. Relative kidney weight increased significantly in low and high-dose females.		Minimum 007871

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TOXCHEM NO. 272E- Cyproconazole FILE LAST PRINTED: 05/14/90

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Feeding/oncogenic-2 year Species: rat Sandoz Ltd 357-R; 4/22/88	SAN 619 F 95.6 +- 1% (Cyproconazole)	411647-01	Levels in diet: 0, 20, 50 & 350 ppm (M - 1.0, 2.2 & 15.6 mg/kg; F - 1.2, 2.7, 21.8 mg/kg) for 118 (M)/121 (F) weeks; decr. body wts in high-dose females & incr. incidence of fatty infiltration of the liver in high dose males; Systemic NOEL = 50 ppm (2.2 mg/kg). Sys. LEL = 350 ppm (15.6 mg/kg). Note: Due to lack of any biologically significant body weight decrement, and any histopathology to correlate accompanying the increased liver weight, any increase or consistent increase in liver enzyme activities in females and males at the high dose, suggests that dose levels were inadequate to determine the carcinogenic potential of SAN 619 F.		Minimum (chronic) 007871 Supp. (Onco) 007871
Dermal-3 week Species: rabbit Sandoz Ltd LMP415-RB; 4/20/88	Cyproconazole tech 95.6%	406243-04	Levels tested in NZW strain - 50, 250 and 1250 mg/kg. NOEL = 250 mg/kg; LEL = 1250 mg/kg (inhibited B.W. gain/food consumption (M), increased AST, increased creatinine, increased cholesterol)		Minimum 007003
Feeding-13 week Species: rat Sandoz Ltd 353/354/R; 4/86	Cyproconazole tech 95.7%	406077-18	Levels tested in Han Wistar strain - 0, 20,80, and 320 ppm (1,4,& 16 mg/kg). NOEL < 1 mg/kg. Changes at 16 mg/kg (HDT): inhibited B.W. gain, increased creatinine, increased sodium, and decreased calcium. Increased creatinine also seen at 1mg/kg(LDT) but not at 4 mg/kg (mid-dose). NOEL = 20 ppm.		Minimum 007003 007907
Feeding-28 day Species: rat Sandoz Ltd I.6158/84; 12/18/86	Cyproconazole tech 95.7%	406243-05	Levels tested in Han Wistar strain - 0, 10, 30, 100, & 1000 ppm (0.5 , 1.5, 5.0, 15 , & 50 mg/kg). NOEL = 5 mg/kg , LEL = 15 mg/kg (elevated ated LDH, increased ab. & rel. liver wt., liver vacuolation, increased rel. testes wt., increased rel. adrenal wt.)		Supplementary 007003
Feeding-13 week Species: dog Res. and Consulting Co.; Switz 6521/86	Cyproconazole tech 95.6%	406077-19	Levels tested in beagles - 0, 20, 100, & 500 ppm(0, 0.8, 4.0, & 20 mg/kg). NOEL = 0.8 mg/kg , LEL = 4 mg/kg (increased ab. liver weight & hepatocytomegaly)		Supplementary 007003
Dermal-3 week Species: rabbit	Cyproconazole WG-40 Formulation		Data requirement waived based on other sufficient dermal toxicity data		007282
Mutagenic-Ames Species: bacteria Hazleton Biotechnologies Corp. E-9528; 9/19/86	SAN 619 F 95.6%	406077-25	No evidence of a mutagenic effect at the histidine locus in any of the S. typhimurium strains (TA98, TA100, TA1535, TA1537, & TA1538) used at dose levels of 1, 5, 10, 100, 500, or 1000 ug/plate either with or without rat S9 mix.		Acceptable 007003

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Mutagenic-unscheduled DNA synt Species: rat hepatocytes Univ Darmstadt LMP 099B; 2/28/85	SAN 619 F 94.4%	406077-27	No indication of increased incorporation of 3H-TdR from exposure to SAN 619F either at a single dose level or as part of a dose-related trend. Dose levels: 0.25, 3.3, 6.6, 10 , & 25 ug/ml. However the highest dose level should have been greater than 25 ug/ml, as there was no indication of a decreased incorporation of 3H-TdR at this level. Also, negative control values were 208.9 dpm, rather than in range of 50 - 150 dpm.		Unacceptable 007003
Mutagenic Species: hamster embryo TechnischeHochschule Darmstadt LMP 099C; 2/26/85	SAN 619F 94.4%	406077-24	No transformation of SHE cells from exposure to SAN 619F for 6 or 48 hrs without S9 activation or as a result of 6-hr exposure to SAN 619 F with S9 activation. Dose levels: 20, 50, 100, & 200 ug/ml. No evidence for cytotoxicity at 200 ug/ml but test material precipitated out at concentrations above 200 ug/ml.		Acceptable 007003
Mutagenic-(HGPRT) Species: cho cells TechnischeHochschule Darmstadt LMP 099A; 2/28/85	SAN 619F 94.4%	406077-26	No indication of mutagenic activity either with or without S9 activation at dose levels of 0, 20, 50, 100 or 200 ug/ml. Test material was soluble only up to 200 ug/ml at which there was little or no evidence of cytotoxicity.		Acceptable 007003
Mutagenic-unscheduled DNA synt Species: rat hepatocytes Microbiological Associates T8028.380; 4/26/88	SAN 619 F 96.2%	406077-29	No indication of an increased level of incorporation of 3H-TdR in rat hepatocytes exposured to SAN 619F at 0.15, 0.5, 1.5, 5, or 15 ug/ml with 18 - 20 hr exposure. Insufficient reporting as to levels of 50, 100 and 150 ug/ml were "Too toxic to be evaluated for UDS," particularly as LDH activities indicated toxicities below 100% (16%, 61%,and 68%, respectively).		Unacceptable 007003
Mutagenic-micronucleus assay Species: mouse Litton Bionetics (Netherlands) ID249215005; 1/10/85	SAN 619F purity not reported	406077-28	No indication of a mutagenic response (a significantly increased incidence of micronucleated polychromatic erythrocytes) at any of the SAN F dose levels (16.7, 55.7 and 167 mg/kg) for any of the scheduled sacrifice times (24, 48, and 72 hrs.)		Unacceptable purity 007003
Mutagenic-mitotic arrest Species: bacteria Litton Bionetics (Netherlands) E-9334; 1/11/85	SAN 619F purity not reported	406243-07	No increased absolute number of cycloheximide-resistant colonies or of an increased incidence of aneuploids among these colonies following overnight exposure to SAN 619F at 10,100, 250, 400, 500, or 550 ug/ml in the presence and absence of S9. Range of doses resulted in no to moderate and nearly complete cytotoxicity. No positive control with S9; no information as to how long was the over-night exposure.		Unacceptable 007003
Mutagenic-micronucleus assay Species: mouse Litton Bionetics Inc. 249.215.005; 1/10/85	Cyproconazole tech 94.4%		No mutagenic response. Doses: 16.7, 55.7 & 167.0 mg/kg. Study may be upgraded to acceptable because the purity of the test material and the supplier of the mice were provided.		Acceptable 007283

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Mutagenic Species: CHO cells Res. and Consulting Co.; Switz 0883/ECC 153; 6/28/88	Cyproconazole 95.6% pure	411587-01	A positive response in the nonactivated and S9 activated assays shows cyproconazole to be clastogenic and that metabolic activation was not required to demonstrate the effect.		Acceptable 007632
Acute oral LD50 Species: rat Sandoz Ltd 265/84; 4/29/84	Cyproconazole tech 95.7%	406077-13	LD50 (M) = 1020 mg/kg , LD50 (F) = 1330 mg/kg	3	Minimum 007003
Acute oral LD50 Species: rat Huntingdon Res. Centre, Eng. 8858D/SNC22/AC; 1/6/88	SAN 619F form 40% ai	406069-05	LD50 (M) = 780 mg/kg , LD50 (F) = 1340 mg/kg	3	Minimum 007003
Acute Dermal LD50 Species: rabbit Sandoz Ltd 6393/85; 7/24/85	Cyproconazole tech 95.7%	406077-13	LD50 > 2000 mg/kg (only dose)	3	Minimum 007003
Acute Dermal LD50 Species: rat Sandoz Ltd 6172/84; 11/15/84	Cyproconazole tech 95.7%	406077-14	LD50 > 2000 mg/kg (only dose)	3	Minimum 007003
Acute Dermal LD50 Species: rat Huntingdon Res. Centre, Eng. 8718210/SNC23/AC; 12/10/87	SAN 619F form 40% ai	406069-06	LD50 > 2000 mg/kg (only dose)	3	Minimum 007003
Acute inhalation LC50 Species: rat Res. and Consulting Co.; Switz 052975; 9/19/85	Cyproconazole tech 95+-1%	406077-15	LC50 > 5.6 mg/l (HDT)	3	Minimum 007003
Primary eye irritation Species: rabbit Huntingdon Res. Centre, Eng. 8817D/SNC17/SE; 12/14/87	Cyproconazole tech (purit y not given)	406077-16	Data are equivocal; study must be repeated.		Supplementary 007003

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS -	TOX CAT	COREGRADE/ DOCUMENT#
Primary eye irritation Species: rabbit Huntingdon Res. Centre, Eng. 8816D/SNC 25?SE; 12/21/87	SAN 619F form 40% ai	406069-07	No primary irritation reaction	4	Minimum 007003
Primary eye irritation Species: rabbit Sandoz Ltd 6322-85; 3/30/85	Cyproconazole tech 94.4%	410427-01	Ocular administration of 100 mg to each of 3 rabbits according to OECD test protocol guidelines. Response to TB evaluation of a previous primary irritation study which had equivocal data; a repeated study was requested. Primary eye irritation was not induced by the test material.	4	Minimum 007325
Primary dermal irritation Species: rabbit Huntingdon Res. Centre, Eng. 871672D/SNC16/SE; 11/27/87	Cyproconazole tech (purity not)	406077-17	No primary irritation reaction	4	Minimum 007003
Primary dermal irritation Species: rabbit Huntingdon Res. Centre, Eng. 871718d/SNC24/SE; 12/4/87	SAN 619F form 40% ai	406069-08	Slight, transient irritation; not evident at 72 hr.	4	Minimum 007003
Dermal sensitization Species: guinea pig Sandoz Ltd 6390/85	Cyproconazole tech 94.4%	406243-04	No sensitization reaction		Minimum 007003
Dermal sensitization Species: guinea pig Huntingdon Res. Centre, Eng. 882598D/SNC/26/SS; 2/29/88	SAN 619F form 40% ai	406069-09	No skin sensitization reaction		Minimum 007003